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(54) Title: DEGRADABLE CLOSTRIDIAL TOXINS

(57) Abstract: The specification discloses modified Clostridial toxins comprising a PAR ligand domain, a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin binding domain; polynucleotide molecules encoding modified Clostridial toxins comprising a PAR ligand domain, a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin binding domain; and method of producing modified Clostridial toxins comprising a PAR ligand domain, a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin binding domain.

WO 2006/026780 A1

Steward, L.E. *et al.*, Degradable Clostridial Toxins

### Degradable Clostridial Toxins

[01] This patent application claims priority pursuant to 35 U.S.C. §119(e) to United States provisional patent application Serial No. 60/651494, which was converted on July 5, 2005 from U.S. nonprovisional patent application Serial No. 10/931719 filed Sep. 1, 2004, which is hereby incorporated by reference in its entirety.

[02] All of the patents and publications cited in this application are hereby incorporated by reference in their entirety.

[03] The ability of Clostridial toxins, such as, *e.g.*, Botulinum neurotoxins (BoNTs), BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F and BoNT/G, and Tetanus neurotoxin (TeNT), to inhibit neuronal transmission are being exploited in a wide variety of therapeutic and cosmetic applications, see *e.g.*, William J. Lipham, *COSMETIC AND CLINICAL APPLICATIONS OF BOTULINUM TOXIN* (Slack, Inc., 2004). As an example, BOTOX® is currently approved in one or more countries for the following indications: achalasia, adult spasticity, anal fissure, back pain, blepharospasm, bruxism, cervical dystonia, essential tremor, glabellar lines or hyperkinetic facial lines, headache, hemifacial spasm, hyperactivity of bladder, hyperhidrosis, juvenile cerebral palsy, multiple sclerosis, myoclonic disorders, nasal labial lines, spasmodic dysphonia, strabismus and VII nerve disorder. In addition, Clostridial toxin therapies are proposed for treating neuromuscular disorders, see *e.g.*, Kei Roger Aoki *et al.*, *Method for Treating Neuromuscular Disorders and Conditions with Botulinum Toxin Types A and B*, U.S. Patent No. 6,872,397 (Mar. 29, 2005); Rhett M. Schiffman, *Methods for Treating Uterine Disorders*, U.S. Patent Publication No. 2004/0175399 (Sep. 9, 2004); Richard L. Barron, *Methods for Treating Ulcers and Gastroesophageal Reflux Disease*, U.S. Patent Publication No. 2004/0086531 (May. 7, 2004); and Kei Roger Aoki, *et al.*, *Method for Treating Dystonia with Botulinum Toxin C to G*, U.S. Patent No. 6,319,505 (Nov. 20, 2001); eye disorders, see *e.g.*, Eric R. First, *Methods and Compositions for Treating Eye Disorders*, U.S. Patent Publication No. 2004/0234532 (Nov. 25, 2004); Kei Roger Aoki *et al.*, *Botulinum Toxin Treatment for Blepharospasm*, U.S. Patent Publication No. 2004/0151740 (Aug. 5, 2004); and Kei Roger Aoki *et al.*, *Botulinum Toxin Treatment for Strabismus*, U.S. Patent Publication No. 2004/0126396 (Jul. 1, 2004); pain, see *e.g.*, Kei Roger Aoki *et al.*, *Pain Treatment by Peripheral Administration of a Neurotoxin*, U.S. Patent No. 6,869,610 (Mar. 22, 2005); Stephen Donovan, *Clostridial Toxin Derivatives and Methods to Treat Pain*, U.S. Patent No. 6,641,820 (Nov. 4, 2003); Kei Roger Aoki, *et al.*, *Method for Treating Pain by Peripheral Administration of a Neurotoxin*, U.S. Patent No. 6,464,986 (Oct. 15, 2002); Kei Roger Aoki and Minglei Cui, *Methods for Treating Pain*, U.S. Patent No. 6,113,915 (Sep. 5, 2000); Martin A. Voet, *Methods for Treating Fibromyalgia*, U.S. Patent 6,623,742 (Sep. 23, 2003); Martin A. Voet, *Botulinum Toxin Therapy for Fibromyalgia*, U.S. Patent Publication No. 2004/0062776 (Apr. 1, 2004); and Kei Roger Aoki *et al.*, *Botulinum Toxin Therapy for Lower Back Pain*, U.S. Patent Publication No. 2004/0037852 (Feb. 26, 2004); muscle injuries, see *e.g.*, Gregory F. Brooks, *Methods for Treating Muscle Injuries*, U.S. Patent No. 6,423,319 (Jul. 23, 2002); headache, see *e.g.*, Martin Voet, *Methods for*

Steward, L.E. *et al.*, Degradable Clostridial Toxins

*Treating Sinus Headache*, U.S. Patent No. 6,838,434 (Jan. 4, 2005); Kei Roger Aoki *et al.*, *Methods for Treating Tension Headache*, U.S. Patent No. 6,776,992 (Aug. 17, 2004); and Kei Roger Aoki *et al.*, *Method for Treating Headache*, U.S. Patent No. 6,458,365 (Oct. 1, 2002); William J. Binder, *Method for Reduction of Migraine Headache Pain*, U.S. Patent 5,714,469 (Feb. 3, 1998); cardiovascular diseases, see *e.g.*, Gregory F. Brooks and Stephen Donovan, *Methods for Treating Cardiovascular Diseases with Botulinum Toxin*, U.S. Patent No. 6,767,544 (Jul. 27, 2004); neurological disorders, see *e.g.*, Stephen Donovan, *Parkinson's Disease Treatment*, U.S. Patent No. 6,620,415 (Sep. 16, 2003); and Stephen Donovan, *Method for Treating Parkinson's Disease with a Botulinum Toxin*, U.S. Patent No. 6,306,403 (Oct. 23, 2001); neuropsychiatric disorders, see *e.g.*, Stephen Donovan, *Botulinum Toxin Therapy for Neuropsychiatric Disorders*, U.S. Patent Publication No. 2004/0180061 (Sep. 16, 2004); and Steven Donovan, *Therapeutic Treatments for Neuropsychiatric Disorders*, U.S. Patent Publication No. 2003/0211121 (Nov. 13, 2003); endocrine disorders, see *e.g.*, Stephen Donovan, *Method for Treating Endocrine Disorders*, U.S. Patent No. 6,827,931 (Dec. 7, 2004); Stephen Donovan, *Method for Treating Thyroid Disorders with a Botulinum Toxin*, U.S. Patent No. 6,740,321 (May. 25, 2004); Kei Roger Aoki *et al.*, *Method for Treating a Cholinergic Influenced Sweat Gland*, U.S. Patent No. 6,683,049 (Jan. 27, 2004); Stephen Donovan, *Neurotoxin Therapy for Diabetes*, U.S. Patent No. 6,416,765 (Jul. 9, 2002); Stephen Donovan, *Methods for Treating Diabetes*, U.S. Patent No. 6,337,075 (Jan. 8, 2002); Stephen Donovan, *Method for Treating a Pancreatic Disorder with a Neurotoxin*, U.S. Patent No. 6,261,572 (Jul. 17, 2001); Stephen Donovan, *Methods for Treating Pancreatic Disorders*, U.S. Patent No. 6,143,306 (Nov. 7, 2000); cancers, see *e.g.*, Stephen Donovan, *Methods for Treating Bone Tumors*, U.S. Patent No. 6,565,870 (May 20, 2003); Stephen Donovan, *Method for Treating Cancer with a Neurotoxin to Improve Patient Function*, U.S. Patent No. 6,368,605 (Apr. 9, 2002); Stephen Donovan, *Method for Treating Cancer with a Neurotoxin*, U.S. Patent No. 6,139,845 (Oct. 31, 2000); and Mitchell F. Brin and Stephen Donovan, *Methods for Treating Diverse Cancers*, U.S. Patent Publication No. 2005/0031648 (Feb. 10, 2005); otic disorders, see *e.g.*, Stephen Donovan, *Neurotoxin Therapy for Inner Ear Disorders*, U.S. Patent No. 6,358,926 (Mar. 19, 2002); and Stephen Donovan, *Method for Treating Otic Disorders*, U.S. Patent No. 6,265,379 (Jul. 24, 2001); autonomic disorders, see, *e.g.*, Pankaj J. Pasricha and Anthony N. Kalloo, *Method for Treating Gastrointestinal Muscle Disorders and Other Smooth Muscle Dysfunction*, U.S. Patent 5,437,291 (Aug. 1, 1995); as well as other disorders, see *e.g.*, William J. Binder, *Method for Treatment of Skin Lesions Associated with Cutaneous Cell-proliferative Disorders*, U.S. Patent 5,670,484 (Sep. 23, 1997); Eric R. First, *Application of Botulinum Toxin to the Management of Neurogenic Inflammatory Disorders*, U.S. Patent 6,063,768 (May 16, 2000); Marvin Schwartz and Brian J. Freund, *Method to Reduce Hair Loss and Stimulate Hair Growth*, U.S. Patent 6,299,893 (Oct. 9, 2001); Jean D. A. Carruthers and Alastair Carruthers, *Cosmetic Use of Botulinum Toxin for Treatment of Downturned Mouth*, U.S. Patent 6,358,917 (Mar. 19, 2002); Stephen Donovan, *Use of a Clostridial Toxin to Reduce Appetite*, U.S. Patent Publication No. 2004/40253274 (Dec. 16, 2004); and Howard I. Katz and Andrew M. Blumenfeld, *Botulinum Toxin Dental Therapies and Procedures*, U.S. Patent Publication No. 2004/0115139 (Jun. 17, 2004); Kei Roger Aoki, *et al.*, *Treatment of Neuromuscular Disorders and Conditions with Different Botulinum*, U.S. Patent Publication No. 2002/0010138 (Jan. 24, 2002); and Kei

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Roger Aoki, et al., *Use of Botulinum Toxins for Treating Various Disorders and Conditions and Associated Pain*, U.S. Patent Publication No. 2004/0013692 (Jan. 22, 2004). In addition, the expected use of Clostridial toxins, such as, *e.g.*, BoNTs and TeNT, in therapeutic and cosmetic treatments of humans and other mammals is anticipated to expand to an ever widening range of diseases and ailments that can benefit from the properties of these toxins.

[04] Clostridial toxin therapies are successfully used for many indications. Generally, administration of a Clostridial toxin is well tolerated. However, toxin administration in some applications can be challenging because of the larger doses required to achieve a beneficial effect. Larger doses can increase the likelihood that the toxin may move through the interstitial fluids and the circulatory systems, such as, *e.g.*, the cardiovascular system and the lymphatic system, of the body, resulting in the undesirable dispersal of the toxin to areas not targeted for toxin treatment. Such dispersal can lead to undesirable side effects, such as, *e.g.*, inhibition of neurotransmitter release in neurons not targeted for treatment or paralysis of a muscle not targeted for treatment. For example, a patient administered a therapeutically effective amount of a BoNT/A treatment into the neck muscles for torticollis may develop dysphagia because of dispersal of the toxin into the oropharynx. Thus, there remains a need for improved Clostridial toxins that are effective at the site of treatment, but have negligible to minimal effects in areas not targeted for a toxin treatment.

[05] The growing clinical, therapeutic and cosmetic use of Clostridial toxins in therapies requiring larger doses necessitates the pharmaceutical industry to develop modified Clostridial toxins that are effective at the target site of the application, but reduce or prevent the undesirable side-effects associated with the dispersal of the toxins to an unwanted location or locations. The present invention provides novel Clostridial toxins that reduce or prevent unwanted side-effects associated with toxin dispersal into non-targeted areas. These and related advantages are useful for various clinical, therapeutic and cosmetic applications, such as, *e.g.*, the treatment of neuromuscular disorders, neuropathic disorders, eye disorders, pain, muscle injuries, headache, cardiovascular diseases, neuropsychiatric disorders, endocrine disorders, cancers, otic disorders and hyperkinetic facial lines, as well as, other disorders where a Clostridial toxin administration to a mammal can produce a beneficial effect.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[06] FIG. 1 shows that activated PARs are predominantly targeted toward lysosomes for degradation. PARs are activated by an irreversible mechanism, and once cleaved, most activated PARs are endocytosed and directed, by intracellular trafficking routes, to lysosomes for degradation. Step 1 illustrates cleavage of an inactivated PAR by a protease to unmask the tethered ligand (black box). Step 2 illustrates tethered ligand binding and conformational change of the activated PAR. Step 3 illustrates endocytosis of the activated PAR. Step 4 illustrates the early and late endosomal sorting of the



Steward, L.E. *et al.*, Degradable Clostridial Toxins

internalized activated PAR that result in the trafficking of the receptor to a lysosome. Step 5 illustrates the degradation of the internalized activated PAR by proteases within the lysosome.

**[07] FIG. 2** shows modified Clostridial toxins comprising a tethered ligand are targeted toward lysosomes for degradation. Such modified toxins that diffuse into the circulatory system can bind to inactive PARs which triggers endocytosis and the directing of internalized toxins, by intracellular trafficking routes, to lysosomes for degradation. Step 1 illustrates the binding of the modified Clostridial toxin comprising a tethered ligand domain (black box) to a PAR. Step 2 illustrates endocytosis of the toxin-PAR complex. Step 3 illustrates the early and late endosomal sorting of the internalized toxin-PAR complex that result in the trafficking of the complex to a lysosome. Step 5 illustrates the degradation of the internalized toxin-PAR complex by proteases within the lysosome.

**[08] FIG. 3** shows a schematic of the current paradigm of neurotransmitter release and Clostridial toxin intoxication in a central and peripheral neuron. FIG. 3a shows a schematic for the neurotransmitter release mechanism of a central and peripheral neuron. The release process can be described as comprising two steps: 1) vesicle docking, where the vesicle-bound SNARE protein of a vesicle containing neurotransmitter molecules associates with the membrane-bound SNARE proteins located at the plasma membrane; and 2) neurotransmitter release, where the vesicle fuses with the plasma membrane and the neurotransmitter molecules are exocytosed. FIG. 3b shows a schematic of the intoxication mechanism for tetanus and botulinum toxin activity in a central and peripheral neuron. This intoxication process can be described as comprising four steps: 1) receptor binding, where a Clostridial toxin binds to a Clostridial receptor system and initiates the intoxication process; 2) complex internalization, where after toxin binding, a vesicle containing the toxin/receptor system complex is endocytosed into the cell; 3) light chain translocation, where multiple events are thought to occur, including, *e.g.*, changes in the internal pH of the vesicle, formation of a channel pore comprising the H<sub>N</sub> domain of the Clostridial toxin heavy chain, separation of the Clostridial toxin light chain from the heavy chain, and release of the active light chain and 4) enzymatic target modification, where the activate light chain of Clostridial toxin proteolytically cleaves its target SNARE substrate, such as, *e.g.*, SNAP-25, VAMP or Syntaxin, thereby preventing vesicle docking and neurotransmitter release.

**[09] FIG. 4** shows modified Clostridial toxins with a PAR ligand domain located at the amino terminus of the enzymatic domain. FIG. 4A depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising a PAR ligand domain, an enzymatic domain, a translocation domain and a binding domain, with the di-chain loop region depicted by the double SS bracket and the resulting di-chain form after di-chain loop cleavage. In this example, a masked PAR ligand domain is located at the amino terminus of the enzymatic domain and a proteolytic cleavage site (P1) is located in front of the PAR ligand binding domain. Upon proteolytic cleavage with a P1 protease, the PAR ligand domain becomes unmasked. P2 is a protease cleavage site used to convert the single chain form of the toxin to the di-chain form. Both P1 and P2 can be a PAR endogenous protease cleavage site or an

Steward, L.E. *et al.*, Degradable Clostridial Toxins

exogenous protease cleavage site and can be cleaved by the same protease or different proteases. FIG. 4B depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising a PAR ligand domain, an enzymatic domain, a binding domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket. In this example, a masked PAR ligand domain is located at the amino terminus of the enzymatic domain and a proteolytic cleavage site (P1) is located in front of the PAR ligand binding domain. Upon proteolytic cleavage with a P1 protease, the PAR ligand domain becomes unmasked. P2 is a protease cleavage site used to convert the single chain form of the toxin to the di-chain form. Both P1 and P2 can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site and can be cleaved by the same protease or different proteases. FIG. 4C depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising a PAR ligand domain, an enzymatic domain, a translocation domain and a binding domain, with the di-chain loop region depicted by the double SS bracket. In this example, an unmasked PAR ligand domain is located at the amino terminus of the enzymatic domain. P2 is a protease cleavage site used to convert the single chain form of the toxin to the di-chain form and can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site. FIG. 4D depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising a PAR ligand domain, an enzymatic domain, a binding domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket. In this example, an unmasked PAR ligand domain is located at the amino terminus of the enzymatic domain. P2 is a protease cleavage site used to convert the single chain form of the toxin to the di-chain form and can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site.

**[010]** FIG. 5 shows modified Clostridial toxins with a PAR ligand domain located at the amino terminus of the translocation domain. FIG. 5A depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising a binding domain, an enzymatic domain, a PAR ligand domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket and the resulting di-chain form after di-chain loop cleavage. In this example, a masked PAR ligand domain is located at the amino terminus of the translocation domain and a proteolytic cleavage site (P1) is located in front of the PAR ligand binding domain. Upon proteolytic cleavage with a P1 protease, the PAR ligand domain becomes unmasked. P1 is also the protease cleavage site used to convert the single chain form of the toxin to the di-chain form. P1 can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site. FIG. 5B depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising an enzymatic domain, a PAR ligand domain, a translocation domain and a binding domain, with the di-chain loop region depicted by the double SS bracket. In this example, a masked PAR ligand domain is located at the amino terminus of the translocation domain and a proteolytic cleavage site (P1) is located in front of the PAR ligand binding domain. Upon proteolytic cleavage with a P1 protease, the PAR ligand domain becomes unmasked. P1 is also the protease cleavage site used to convert the single chain form of the toxin to the di-chain form. P1 can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site.

[011] FIG. 6 shows modified Clostridial toxins with a PAR ligand domain located at the amino terminus of the binding domain. FIG. 6 depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising an enzymatic domain, a PAR ligand domain, a binding domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket and the resulting di-chain form after di-chain loop cleavage. In this example, a masked PAR ligand domain is located at the amino terminus of the binding domain and a proteolytic cleavage site (P1) is located in front of the PAR ligand binding domain. Upon proteolytic cleavage with a P1 protease, the PAR ligand domain becomes unmasked. P1 is also the protease cleavage site used to convert the single chain form of the toxin to the di-chain form. P1 can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site.

[012] FIG. 7 shows a plasmid map of prokaryotic expression construct pET29b/BoNT/A-ED-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 136 encoding a modified BoNT/A of SEQ ID NO: 85, operably-linked to a carboxyl-terminal polyhistidine binding polypeptide. A Trypsin protease cleavage site is operably-linked between the polyhistidine binding polypeptide and the modified BoNT/A. Abbreviations are as follows: P<sub>T7</sub>, a bacteriophage T7 promoter region; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain; Trypsin, a polynucleotide molecule encoding Trypsin cleavage site; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; T7 TT, a bacteriophage T7 transcription termination region; f1 origin, a bacteriophage f1 origin of replication; Kanamycin, a polynucleotide molecule encoding an aminophosphotransferase that confers Kanamycin resistance; pBR322 ori, a pBR322 origin of plasmid replication region; lacI, a polynucleotide molecule encoding a lactose I.

[013] FIG. 8 shows a plasmid map of prokaryotic expression construct pET29b/BoNT/A-TD-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 144 encoding a modified BoNT/A of SEQ ID NO: 93, operably-linked to a carboxyl-terminal polyhistidine binding polypeptide. A Trypsin protease cleavage site is operably-linked between the polyhistidine binding polypeptide and the modified BoNT/A. Abbreviations are as follows: P<sub>T7</sub>, a bacteriophage T7 promoter region; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain; Trypsin, a polynucleotide molecule encoding Trypsin cleavage site; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; T7 TT, a bacteriophage T7 transcription termination region; f1 origin, a bacteriophage f1 origin of replication; Kanamycin, a polynucleotide molecule encoding an aminophosphotransferase that confers Kanamycin resistance;

Steward, L.E. *et al.*, Degradable Clostridial Toxins

pBR322 ori, a pBR322 origin of plasmid replication region; lacI, a polynucleotide molecule encoding a lactose I.

**[014]** FIG. 9 shows a plasmid map of prokaryotic expression construct pET29b/BoNT/A-BD-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 152 encoding a modified BoNT/A of SEQ ID NO: 101, operably-linked to a carboxyl-terminal polyhistidine binding polypeptide. A Trypsin protease cleavage site is operably-linked between the polyhistidine binding polypeptide and the modified BoNT/A. Abbreviations are as follows: P<sub>T7</sub>, a bacteriophage T7 promoter region; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain; Trypsin, a polynucleotide molecule encoding Trypsin cleavage site; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; T7 TT, a bacteriophage T7 transcription termination region; f1 origin, a bacteriophage f1 origin of replication; Kanamycin, a polynucleotide molecule encoding an aminophosphotransferase that confers Kanamycin resistance; pBR322 ori, a pBR322 origin of plasmid replication region; lacI, a polynucleotide molecule encoding a lactose I.

**[015]** FIG. 10 shows a plasmid map of yeast expression construct pPICZ A/BoNT/A-ED-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 136 encoding a modified BoNT/A of SEQ ID NO: 85, operably-linked to carboxyl-terminal c-myc and polyhistidine binding polypeptides. Abbreviations are as follows: P<sub>AOX1</sub>, an aldehyde oxidase 1 promoter region; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain; c-myc, a polynucleotide molecule encoding a c-myc binding polypeptide; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; AOX1 TT, an aldehyde oxidase 1 transcription termination region; Zeocin™, a polynucleotide molecule encoding a Zeocin™ resistance polypeptide; pUC ori, a pUC origin of plasmid replication region.

**[016]** FIG. 11 shows a plasmid map of baculovirus transfer construct pBACgus3/BoNT/A-ED-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 136 encoding a modified BoNT/A of SEQ ID NO: 85, operably-linked to carboxyl-terminal polyhistidine binding polypeptide. A Thrombin protease cleavage site is operably-linked between the modified BoNT/A and the polyhistidine binding polypeptide. Abbreviations are as follows: P<sub>PH</sub>, an polyhedrin promoter region; gp64, a polynucleotide molecule encoding a gp64 signal polypeptide; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain;

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Thrombin, a polynucleotide molecule encoding a Thrombin protease cleavage site; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; pUC ori, a pUC origin of plasmid replication region; Ampicillin, a polynucleotide molecule encoding a  $\beta$ -lactamase that confers Ampicillin resistance; f1 ori, a bacteriophage f1 origin of replication; gus, a polynucleotide molecule encoding a  $\beta$ -glucuronidase.

[017] FIG. 12 shows a plasmid map of mammalian expression construct pSecTag2/BoNT/A-ED-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 136 encoding a modified BoNT/A of SEQ ID NO: 85, operably-linked to carboxyl-terminal c-myc and polyhistidine binding polypeptides. Abbreviations are as follows: P<sub>CMV</sub>, an cytomegalovirus promoter region; IgK, a polynucleotide molecule encoding an immunoglobulin K polypeptide; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain; c-myc, a polynucleotide molecule encoding a c-myc binding polypeptide; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; BGH pA, a bovine growth hormone polyadenylation site; f1 ori, a bacteriophage f1 origin of replication; P<sub>SV40</sub>, a simian virus 40 promoter region; Zeocin™, a region encoding an Zeocin™ resistance polypeptide; pUC ori, a pUC origin of plasmid replication region; Ampicillin, a polynucleotide molecule encoding a  $\beta$ -lactamase that confers Ampicillin resistance.

## DETAILED DESCRIPTION

[018] While all details of this process are not yet precisely known, protease-activated G protein-coupled receptor (PAR) signaling elicits responses according to the classic paradigm established for other GPCRs. Although the applicants have no wish to be limited by the following description, the overall signaling mechanism can be described as comprising at least four steps: 1) receptor activation where the protease agonist cleaves a specific site located at the extracellular amino-terminus of the receptor that generates a new amino acid terminus that functions as a tethered ligand; 2) ligand binding where the unmasked tethered ligand binds to the ligand binding domain located in the second extracellular loop of the receptor resulting in a conformational change of the cleaved PAR that promotes intracellular interactions with heteromeric G proteins; 3) signal transduction where, in common with most GPCRs, the PAR-G protein complex signals through various Gq-, Gi- and G $\beta\gamma$ -mediated signaling pathways in a temporal and spatial manner; and 4) signal termination where receptor desensitization and receptor degradation stop the signaling of the activated complex (FIG. 1), see, *e.g.*, Joann Trejo, *Protease-Activated Receptors: New Concepts in Regulation of G Protein-Coupled Receptor Signaling and Trafficking*, 307(2) J. Pharmacol. Exp. Ther. 437-442 (2003); and Valeria S. Ossovskaya and Nigel W. Bennett, *Protease-Activated Receptors: Contribution to Physiology and Disease*, 84(2) Physiol. Rev. 579-621 (2004).

Steward, L.E. *et al.*, Degradable Clostridial Toxins

**[019]** Despite the irreversible mechanism of receptor activation, signaling initiated by activated PARs appears to be rapidly and efficiently terminated. Signal termination is especially important for regulating the magnitude, duration and fidelity of PAR-elicited cellular responses and appears to be governed by two processes. The first mechanism is receptor desensitization, where enzymatic phosphorylation of the activated PAR by G-protein Receptor Kinases (GRKs) and other kinases uncouple the activated receptor from its associated G proteins and signaling effectors. The second mechanism of PAR-initiated signal termination is receptor degradation, where proteolytic cleavage of the activated PAR by cell-surface proteases on the plasma membrane and by intracellular proteases within lysosomal vesicles destroys the activated receptors. Because of the irreversible nature of PAR activation, internalization of activated PARs and their subsequent sorting to lysosomes appears to be the dominant process for signal termination. Internalization of activated PARs contributes to signal termination both by removing activated receptors from G proteins and signaling effectors and by directing activated receptors to lysosomal vesicles where proteolytic degradation effectively inactivates the activated receptor. In addition to endocytosis of activated receptors, PARs also undergo constitutive endocytosis in the absence of proteolytic activation. Therefore, the unusual and irreversible mode of PAR activation has given rise to a very rapid and efficient means of terminating the signaling events elicited by activated PARs utilizing endocytosis and lysosomal degradation.

**[020]** The present invention discloses modified Clostridial toxins that can be rapidly removed from the circulatory system by exploiting the processes involved in activated PAR signal termination. Clostridial toxins containing a PAR ligand domain can bind PARs, which initiates the internalization and degradation of such modified toxins. Many tissues of the cardiovascular system and lymphatic system comprise cells which express PARs. In situations where a modified Clostridial toxin comprising a PAR ligand domain has diffused into a circulatory system, this modified toxin can be effectively internalized by a PAR expressing cell and degraded by proteases within lysosomes (FIG. 2). Thus utilizing the processes involved in PAR-elicited signal termination will lessen or remove a Clostridial toxin from the circulatory system thereby reducing or preventing the undesirable side-effects associated with the diffusion of a Clostridial toxin to an unwanted location.

**[021]** Aspects of the present invention provide modified Clostridial toxins comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain. It is envisioned that the location of the PAR ligand domain in the modified Clostridial toxins of the present specification is located at a free amino terminus, including, without limitation, at the amino terminus of the Clostridial toxin enzymatic domain; at the amino terminus of the Clostridial toxin translocation domain; and at the amino terminus of the Clostridial toxin binding domain. Thus, in embodiments, the modified Clostridial toxins comprise a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin enzymatic domain. In other embodiments, the modified Clostridial toxins comprise a PAR ligand domain; a

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin translocation domain. In still other embodiments, the modified Clostridial toxins comprise a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin binding domain.

**[022]** Other aspects of the present invention provide polynucleotide molecules encoding modified Clostridial toxins comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain. It is envisioned that the location of the PAR ligand domain of the modified Clostridial toxins encoded by polynucleotide molecules of the present specification is located at a free amino terminus, including, without limitation, at the amino terminus of the Clostridial toxin enzymatic domain; at the amino terminus of the Clostridial toxin translocation domain; and at the amino terminus of the Clostridial toxin binding domain. Thus, in embodiments, the polynucleotide molecules encoded modified Clostridial toxins comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin enzymatic domain. In other embodiments, the polynucleotide molecules encoded modified Clostridial toxins comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin translocation domain. In still other embodiments, the polynucleotide molecules encoded modified Clostridial toxins comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin binding domain.

**[023]** Other aspects of the present invention provide methods of producing a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain, such method comprising the step of expressing in a cell a polynucleotide molecule encoding a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain. Other aspects of the present invention provide methods of producing in a cell a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain, such method comprising the steps of introducing in a cell an expression construct comprising a polynucleotide molecule encoding a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain and expressing the expression construct in the cell.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

[024] Aspects of the present invention provide, in part, a Clostridial toxin. As used herein, the term "Clostridial toxin" means any polypeptide that can execute the overall cellular mechanism whereby a Clostridial toxin enters a neuron and inhibits neurotransmitter release and encompasses the binding of a Clostridial toxin to a low or high affinity receptor complex, the internalization of the toxin/receptor complex, the translocation of the Clostridial toxin light chain into the cytoplasm and the enzymatic modification of a Clostridial toxin substrate. Clostridia toxins produced by *Clostridium botulinum*, *Clostridium tetani*, *Clostridium baratii* and *Clostridium butyricum* are the most widely used in therapeutic and cosmetic treatments of humans and other mammals. Strains of *C. botulinum* produce seven antigenically-distinct types of Botulinum toxins (BoNTs), which have been identified by investigating botulism outbreaks in man (BoNT/A, /B, /E and /F), animals (BoNT/C1 and /D), or isolated from soil (BoNT/G). BoNTs possess approximately 35% amino acid identity with each other and share the same functional domain organization and overall structural architecture. It is recognized by those of skill in the art that within each type of Clostridial toxin there can be subtypes that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently four BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4, with specific subtypes showing approximately 89% amino acid identity when compared to another BoNT/A subtype. While all seven BoNT serotypes have similar structure and pharmacological properties, each also displays heterogeneous bacteriological characteristics. In contrast, tetanus toxin (TeNT) is produced by a uniform group of *C. tetani*. Two other species of Clostridia, *C. baratii* and *C. butyricum*, also produce toxins similar to BoNT/F and BoNT/E, respectively.

[025] Clostridial toxins are each translated as a single chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease, such as, *e.g.*, an endogenous Clostridial toxin protease or a naturally-occurring proteases produced in the environment. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulfide bond and noncovalent interactions. Each mature di-chain molecule comprises three functionally distinct domains: 1) an enzymatic domain located in the LC that includes a metalloprotease region containing a zinc-dependent endopeptidase activity which specifically targets core components of the neurotransmitter release apparatus (Table 1); 2) a translocation domain contained within the amino-terminal half of the HC ( $H_N$ ) that facilitates release of the LC from intracellular vesicles into the cytoplasm of the target cell (Table 1); and 3) a binding domain found within the carboxyl-terminal half of the HC ( $H_C$ ) that determines the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell (Table 1).

[026] The binding, translocation and enzymatic activity of these three functional domains are all necessary for toxicity. While all details of this process are not yet precisely known, the overall cellular intoxication mechanism whereby Clostridial toxins enter a neuron and inhibit neurotransmitter release is similar, regardless of type. Although the applicants have no wish to be limited by the following description, the intoxication mechanism can be described as comprising at least four steps: 1) receptor



Steward, L.E. *et al.*, Degradable Clostridial Toxins

binding, 2) complex internalization, 3) light chain translocation, and 4) enzymatic target modification (see FIG. 1). The process is initiated when the H<sub>C</sub> domain of a Clostridial toxin binds to a toxin-specific receptor complex located on the plasma membrane surface of a target cell. The binding specificity of a receptor complex is thought to be achieved, in part, by specific combinations of gangliosides and protein receptors that appear to distinctly comprise each Clostridial toxin receptor complex. Once bound, the toxin/receptor complexes are internalized by endocytosis and the internalized vesicles are sorted to specific intracellular routes. The translocation step appears to be triggered by the acidification of the vesicle compartment. This process seems to initiate two important pH-dependent structural rearrangements that increase hydrophobicity and promote formation di-chain form of the toxin. Once activated, light chain endopeptidase of the toxin is released from the intracellular vesicle into the cytosol where it specifically targets one of three known core components of the neurotransmitter release apparatus. These core proteins, vesicle-associated membrane protein (VAMP)/synaptobrevin, synaptosomal-associated protein of 25 kDa (SNAP-25) and Syntaxin, are necessary for synaptic vesicle docking and fusion at the nerve terminal and constitute members of the soluble *N*-ethylmaleimide-sensitive factor-attachment protein-receptor (SNARE) family. BoNT/A and BoNT/E cleave SNAP-25 in the carboxyl-terminal region, releasing a nine or twenty-six amino acid segment, respectively, and BoNT/C1 also cleaves SNAP-25 near the carboxyl-terminus. The botulinum serotypes BoNT/B, BoNT/D, BoNT/F and BoNT/G, and tetanus toxin, act on the conserved central portion of VAMP, and release the amino-terminal portion of VAMP into the cytosol. BoNT/C1 cleaves syntaxin at a single site near the cytosolic membrane surface. The selective proteolysis of synaptic SNAREs accounts for the block of neurotransmitter release caused by Clostridial toxins *in vivo*. The SNARE protein targets of Clostridial toxins are common to exocytosis in a variety of non-neuronal types; in these cells, as in neurons, light chain peptidase activity inhibits exocytosis, see, *e.g.*, Yann Humeau et al., *How Botulinum and Tetanus Neurotoxins Block Neurotransmitter Release*, 82(5) *Biochimie*. 427-446 (2000); Kathryn Turton et al., *Botulinum and Tetanus Neurotoxins: Structure, Function and Therapeutic Utility*, 27(11) *Trends Biochem. Sci.* 552-558. (2002); Giovanna Lalli et al., *The Journey of Tetanus and Botulinum Neurotoxins in Neurons*, 11(9) *Trends Microbiol.* 431-437, (2003).

Table 1. Clostridial Toxin Reference Sequences and Regions				
Toxin	SEQ ID NO:	LC	H <sub>N</sub>	H <sub>C</sub>
BoNT/A	1	M1-K448	A449-K871	N872-L1296
BoNT/B	2	M1-K441	A442-S858	E859-E1291
BoNT/C1	3	M1-K449	T450-N866	N867-E1291
BoNT/D	4	M1-R445	D446-N862	S863-E1276
BoNT/E	5	M1-R422	K423-K845	R846-K1252
BoNT/F	6	M1-K439	A440-K864	K865-E1274
BoNT/G	7	M1-K446	S447-S863	N864-E1297
TeNT	8	M1-A457	S458-V879	I880-D1315

Steward, L.E. *et al.*, Degradable Clostridial Toxins

[027] A Clostridial toxin includes, without limitation, naturally occurring Clostridial toxin variants, such as, *e.g.*, Clostridial toxin isoforms and Clostridial toxin subtypes; non-naturally occurring Clostridial toxin variants, such as, *e.g.*, conservative Clostridial toxin variants, non-conservative Clostridial toxin variants, Clostridial toxin chimeric variants and active Clostridial toxin fragments thereof, or any combination thereof. As used herein, the term "Clostridial toxin variant," whether naturally-occurring or non-naturally-occurring, means a Clostridial toxin that has at least one amino acid change from the corresponding region of the disclosed reference sequences (see Table 1) and can be described in percent identity to the corresponding region of that reference sequence. As non-limiting examples, a BoNT/A variant comprising amino acids 1-1296 of SEQ ID NO: 1 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1296 of SEQ ID NO: 1; a BoNT/B variant comprising amino acids 1-1291 of SEQ ID NO: 2 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1291 of SEQ ID NO: 2; a BoNT/C1 variant comprising amino acids 1-1291 of SEQ ID NO: 3 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1291 of SEQ ID NO: 3; a BoNT/D variant comprising amino acids 1-1276 of SEQ ID NO: 4 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1276 of SEQ ID NO: 4; a BoNT/E variant comprising amino acids 1-1252 of SEQ ID NO: 5 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1252 of SEQ ID NO: 5; a BoNT/F variant comprising amino acids 1-1274 of SEQ ID NO: 6 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1274 of SEQ ID NO: 6; a BoNT/G variant comprising amino acids 1-1297 of SEQ ID NO: 7 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1297 of SEQ ID NO: 7; and a TeNT variant comprising amino acids 1-1315 of SEQ ID NO: 8 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1315 of SEQ ID NO: 8.

[028] Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[029] Global methods align sequences from the beginning to the end of the molecule and determine the best alignment by adding up scores of individual residue pairs and by imposing gap penalties. Non-limiting methods include, *e.g.*, CLUSTAL W, see, *e.g.*, Julie D. Thompson et al., *CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice*, 22(22) Nucleic Acids Research 4673-4680 (1994); and

Steward, L.E. *et al.*, Degradable Clostridial Toxins

iterative refinement, see, *e.g.*, Osamu Gotoh, *Significant Improvement in Accuracy of Multiple Protein Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments*, 264(4) J. Mol. Biol. 823-838 (1996).

**[030]** Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, *e.g.*, Match-box, see, *e.g.*, Eric Depiereux and Ernest Feytmans, *Match-Box: A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences*, 8(5) CABIOS 501-509 (1992); Gibbs sampling, see, *e.g.*, C. E. Lawrence et al., *Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment*, 262(5131) Science 208-214 (1993); Align-M, see, *e.g.*, Ivo Van Walle et al., *Align-M – A New Algorithm for Multiple Alignment of Highly Divergent Sequences*, 20(9) Bioinformatics, 1428-1435 (2004).

**[031]** Hybrid methods combine functional aspects of both global and local alignment methods. Non-limiting methods include, *e.g.*, segment-to-segment comparison, see, *e.g.*, Burkhard Morgenstern et al., *Multiple DNA and Protein Sequence Alignment Based On Segment-To-Segment Comparison*, 93(22) Proc. Natl. Acad. Sci. U.S.A. 12098-12103 (1996); T-Coffee, see, *e.g.*, Cédric Notredame et al., *T-Coffee: A Novel Algorithm for Multiple Sequence Alignment*, 302(1) J. Mol. Biol. 205-217 (2000); MUSCLE, see, *e.g.*, Robert C. Edgar, *MUSCLE: Multiple Sequence Alignment With High Score Accuracy and High Throughput*, 32(5) Nucleic Acids Res. 1792-1797 (2004); and DIALIGN-T, see, *e.g.*, Amarendran R Subramanian et al., *DIALIGN-T: An Improved Algorithm for Segment-Based Multiple Sequence Alignment*, 6(1) BMC Bioinformatics 66 (2005).

**[032]** As used herein, the term “naturally occurring Clostridial toxin variant” means any Clostridial toxin produced without the aid of any human manipulation, including, without limitation, Clostridial toxin isoforms produced from alternatively-spliced transcripts, Clostridial toxin isoforms produced by spontaneous mutation and Clostridial toxin subtypes. Non-limiting examples of a Clostridial toxin isoform include, *e.g.*, BoNT/A isoforms, BoNT/B isoforms, BoNT/C1 isoforms, BoNT/D isoforms, BoNT/E isoforms, BoNT/F isoforms, BoNT/G isoforms, and TeNT isoforms. Non-limiting examples of a Clostridial toxin subtype include, *e.g.*, BoNT/A subtypes BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4; BoNT/B subtypes BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; BoNT/C1 subtypes BoNT/C1-1 and BoNT/C1-2; BoNT/E subtypes BoNT/E1, BoNT/E2 and BoNT/E3; and BoNT/F subtypes BoNT/F1, BoNT/F2, BoNT/F3 and BoNT/F4.

**[033]** As used herein, the term “non-naturally occurring Clostridial toxin variant” means any Clostridial toxin produced with the aid of human manipulation, including, without limitation, Clostridial toxins produced by genetic engineering using random mutagenesis or rational design and Clostridial toxins produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin variants include, *e.g.*, conservative Clostridial toxin variants, non-conservative Clostridial toxin variants, Clostridial toxin chimeric variants and active Clostridial toxin fragments.

**[034]** As used herein, the term “conservative Clostridial toxin variant” means a Clostridial toxin that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin variant can function in substantially the same manner as the reference Clostridial toxin on which the conservative Clostridial toxin variant is based, and can be substituted for the reference Clostridial toxin in any aspect of the present invention. A conservative Clostridial toxin variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin on which the conservative Clostridial toxin variant is based. A conservative Clostridial toxin variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin on which the conservative Clostridial toxin variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin on which the conservative Clostridial toxin variant is based. Non-limiting examples of a conservative Clostridial toxin variant include, *e.g.*, conservative BoNT/A variants, conservative BoNT/B variants, conservative BoNT/C1 variants, conservative BoNT/D variants, conservative BoNT/E variants, conservative BoNT/F variants, conservative BoNT/G variants, and conservative TeNT variants.

**[035]** As used herein, the term “non-conservative Clostridial toxin variant” means a Clostridial toxin in which 1) at least one amino acid is deleted from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based; 2) at least one amino acid added to the reference Clostridial toxin on which the non-conservative Clostridial toxin is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin sequence (Table 1). A non-conservative Clostridial toxin variant can function in substantially the same manner as the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based, and can be substituted for the reference Clostridial toxin in any aspect of the present invention. A non-conservative Clostridial toxin variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. A non-conservative Clostridial toxin variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. A non-conservative

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Clostridial toxin variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. A non-conservative Clostridial toxin variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. Non-limiting examples of a non-conservative Clostridial toxin variant include, *e.g.*, non-conservative BoNT/A variants, non-conservative BoNT/B variants, non-conservative BoNT/C1 variants, non-conservative BoNT/D variants, non-conservative BoNT/E variants, non-conservative BoNT/F variants, non-conservative BoNT/G variants, and non-conservative TeNT variants.

[036] As used herein, the term "Clostridial toxin chimeric variant" means a molecule comprising at least a portion of a Clostridial toxin and at least a portion of at least one other protein to form a toxin with at least one property different from the reference Clostridial toxins of Table 1. Such Clostridial toxin chimeric molecules are described in, *e.g.*, Clifford C. Shone *et al.*, Recombinant Toxin Fragments, US 6,461,617 (Oct. 8, 2002); Keith A. Foster *et al.*, Clostridial Toxin Derivatives Able To Modify Peripheral Sensory Afferent Functions, US 6,395,513 (May 28, 2002); Wei-Jin Lin *et al.*, Neurotoxins with Enhanced Target Specificity, US 2002/0137886 (Sep. 26, 2002); Keith A. Foster *et al.*, Inhibition of Secretion from Non-neural Cells, US 2003/0180289 (Sep. 25, 2003); J. Oliver Dolly *et al.*, Activatable Recombinant Neurotoxins, WO 2001/014570 (Mar. 1, 2001); Clifford C. Shone *et al.*, Recombinant Toxin Fragments, WO 2004/024909 (Mar. 25, 2004); and Keith A. Foster *et al.*, Re-targeted Toxin Conjugates, WO 2005/023309 (Mar. 17, 2005).

[037] It is well documented that toxin molecules can be re-targeted to a cell that is not the toxins' natural target cell. When so re-targeted, these toxins are capable of binding to a desired target cell and, following subsequent translocation into the cytosol, are capable of exerting their effect on the target cell. In this regard, the binding domain is selected so that it will bind to a desired target cell, and allow subsequent passage of the modified Clostridial toxin into an endosome within the target cell. It is envisioned that any non-Clostridial binding domain can be used, including, without limitation, ligands, hormones, growth factors, cytokines, antibodies, antagonists, agonists and reverse-agonists, with the proviso that the non-Clostridial binding domain binds to a cell surface receptor system other than the one used by the Clostridial binding domain of the modified Clostridial toxin. Non-limiting examples of a non-Clostridial binding domain include, growth factors, such as, *e.g.*, Nerve growth factor (NGF), Leukemia inhibitory factor (LIF), Basic fibroblast growth factor (bFGF), Brain-derived neurotrophic factor (BDNF),

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Neurotrophin-3 (NT-3), Hydra head activator peptide (HHAP), Transforming growth factor 1 (TGF-1), Transforming growth factor 2 (TGF-2), Transforming growth factor 3 (TGF-3), Epidermal growth factor (EGF) and Ciliary neurotrophic factor (CNTF); cytokines, such as, *e.g.*, Tumor necrosis factor (TNF-), Interleukin-1 (IL-1), Interleukin-1 (IL-1) and Interleukin-8 (IL-8); agonists, such as, *e.g.*, Bradykinin, Dynorphin,  $\beta$ -endorphin, Etorphine, Endomorphin-1, Endomorphin-2, Leu-enkephalin, Met-enkephalin, Galanin, Lofentanil, Nociceptin and an opioid; and antibodies, such as, *e.g.*, antibodies against the lactoseries carbohydrate epitopes found on the surface of dorsal root ganglion neurons (*e.g.* monoclonal antibodies 1B2 and LA4), antibodies against any of the receptors for the ligands given above and antibodies against the surface expressed antigen Thyl (*e.g.* monoclonal antibody MRC OX7). Methods of making and using a Clostridial toxin chimeric variant can comprise a modified Clostridial toxin disclosed in the present specification where the binding domain comprises a non-Clostridial toxin binding domain are described in, *e.g.*, Clifford C. Shone *et al.*, *supra*, (2002); Keith A. Foster *et al.*, *supra*, (2002); Wei-Jin Lin *et al.*, *supra*, (2002); Keith A. Foster *et al.*, *supra*, (2003); J. Oliver Dolly *et al.*, *supra*, (2001); Clifford C. Shone *et al.*, *supra*, (2004); and Keith A. Foster *et al.*, *supra*, (2005).

**[038]** Thus, in an embodiment, a Clostridial toxin chimeric variant can comprise a modified Clostridial toxin disclosed in the present specification where the binding domain comprises a non-Clostridial toxin binding domain. In aspects of this embodiment, a non-Clostridial toxin binding domain can be, *e.g.*, a ligand, a hormone, a growth factor, a cytokine, an antibody, an opioid, an antagonist, an agonist or a reverse-agonist. In other aspects of this embodiment, a non-Clostridial toxin binding domain is a Nerve growth factor (NGF), a Leukemia inhibitory factor (LIF), a Basic fibroblast growth factor (bFGF), a Brain-derived neurotrophic factor (BDNF), a Neurotrophin-3 (NT-3), a Hydra head activator peptide (HHAP), a Transforming growth factor 1 (TGF-1), a Transforming growth factor 2 (TGF-2), a Transforming growth factor 3 (TGF-3), an Epidermal growth factor (EGF) or a Ciliary neurotrophic factor (CNTF). In still other aspects of this embodiment, a non-Clostridial toxin binding domain is a Tumor necrosis factor (TNF-), an Interleukin-1 (IL-1), an Interleukin-1 (IL-1) or an Interleukin-8 (IL-8). In yet other aspects of this embodiment, a non-Clostridial toxin binding domain is a Bradykinin, a Dynorphin, a  $\beta$ -endorphin, an Etorphine, an Endomorphin-1, an Endomorphin-2, a Leu-enkephalin, a Met-enkephalin, a Galanin, a Lofentanil or a Nociceptin. In still other aspects of this embodiment, a non-Clostridial toxin binding domain is an antibody against the lactoseries carbohydrate epitopes found on the surface of dorsal root ganglion neurons (*e.g.* monoclonal antibodies 1B2 and LA4), an antibody against any of the receptors for the binding domains given above or an antibody against the surface expressed antigen Thyl (*e.g.* monoclonal antibody MRC OX7).

**[039]** It is also envisioned that any of a variety of Clostridial toxin fragments can be useful in aspects of the present invention with the proviso that these active fragments can execute the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. Thus, aspects of this embodiment can include Clostridial toxin fragments having a length of, *e.g.*, at least 300 amino acids, at least 400 amino acids, at least 500 amino acids, at least 600 amino acids, at least 700 amino acids, at

Steward, L.E. *et al.*, Degradable Clostridial Toxins

least 800 amino acids, at least 900 amino acids, at least 1000 amino acids, at least 1100 amino acids and at least 1200 amino acids. Other aspects of this embodiment, can include Clostridial toxin fragments having a length of, *e.g.*, at most 300 amino acids, at most 400 amino acids, at most 500 amino acids, at most 600 amino acids, at most 700 amino acids, at most 800 amino acids, at most 900 amino acids, at most 1000 amino acids, at most 1100 amino acids and at most 1200 amino acids.

**[040]** It is also envisioned that any of a variety of Clostridial toxin fragments comprising the light chain can be useful in aspects of the present invention with the proviso that these light chain fragments can specifically target the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The light chains of Clostridial toxins are approximately 420-460 amino acids in length and comprise an enzymatic domain (Table 1). Research has shown that the entire length of a Clostridial toxin light chain is not necessary for the enzymatic activity of the enzymatic domain. As a non-limiting example, the first eight amino acids of the BoNT/A light chain (residues 1-8 of SEQ ID NO: 1) are not required for enzymatic activity. As another non-limiting example, the first eight amino acids of the TeNT light chain (residues 1-8 of SEQ ID NO: 8) are not required for enzymatic activity. Likewise, the carboxyl-terminus of the light chain is not necessary for activity. As a non-limiting example, the last 32 amino acids of the BoNT/A light chain (residues 417-448 of SEQ ID NO: 1) are not required for enzymatic activity. As another non-limiting example, the last 31 amino acids of the TeNT light chain (residues 427-457 of SEQ ID NO: 8) are not required for enzymatic activity. Thus, aspects of this embodiment can include Clostridial toxin light chains comprising an enzymatic domain having a length of, *e.g.*, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids, at least 425 amino acids and at least 450 amino acids. Other aspects of this embodiment can include Clostridial toxin light chains comprising an enzymatic domain having a length of, *e.g.*, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids, at most 425 amino acids and at most 450 amino acids.

**[041]** It is also envisioned that any of a variety of Clostridial toxin H<sub>N</sub> regions comprising a translocation domain can be useful in aspects of the present invention with the proviso that these active fragments can facilitate the release of the LC from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The H<sub>N</sub> regions from the heavy chains of Clostridial toxins are approximately 410-430 amino acids in length and comprise a translocation domain (Table 1). Research has shown that the entire length of a H<sub>N</sub> region from a Clostridial toxin heavy chain is not necessary for the translocating activity of the translocation domain. Thus, aspects of this embodiment can include Clostridial toxin H<sub>N</sub> regions comprising a translocation domain having a length of, *e.g.*, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids and at least 425 amino acids. Other aspects of this embodiment can include Clostridial toxin H<sub>N</sub> regions comprising translocation domain having a length of, *e.g.*, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids and at most 425 amino acids.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

[042] It is also envisioned that any of a variety of Clostridial toxin H<sub>C</sub> regions comprising a binding domain can be useful in aspects of the present invention with the proviso that these active fragments can determine the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell execute the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The H<sub>C</sub> regions from the heavy chains of Clostridial toxins are approximately 400-440 amino acids in length and comprise a binding domain (Table 1). Research has shown that the entire length of a H<sub>C</sub> region from a Clostridial toxin heavy chain is not necessary for the binding activity of the binding domain. Thus, aspects of this embodiment can include Clostridial toxin H<sub>C</sub> regions comprising a binding domain having a length of, *e.g.*, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids and at least 425 amino acids. Other aspects of this embodiment can include Clostridial toxin H<sub>C</sub> regions comprising a binding domain having a length of, *e.g.*, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids and at most 425 amino acids.

[043] Thus, in an embodiment, a Clostridial toxin comprises a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin binding domain. In an aspect of this embodiment, a Clostridial toxin comprises a naturally occurring Clostridial toxin variant, such as, *e.g.*, a Clostridial toxin isoform or a Clostridial toxin subtype. In another aspect of this embodiment, a Clostridial toxin comprises a non-naturally occurring Clostridial toxin variant, such as, *e.g.*, a conservative Clostridial toxin variant, a non-conservative Clostridial toxin variant or an active Clostridial toxin fragment, or any combination thereof. In another aspect of this embodiment, a Clostridial toxin comprises a Clostridial toxin enzymatic domain or an active fragment thereof, a Clostridial toxin translocation domain or an active fragment thereof, a Clostridial toxin binding domain or an active fragment thereof, or any combination thereof. In other aspects of this embodiment, a Clostridial toxin can comprise a BoNT/A, a BoNT/B, a BoNT/C1, a BoNT/D, a BoNT/E, a BoNT/F, a BoNT/G or a TeNT.

[044] In another embodiment, a Clostridial toxin comprises a BoNT/A. In an aspect of this embodiment, a BoNT/A comprises a BoNT/A enzymatic domain, a BoNT/A translocation domain and a BoNT/A binding domain. In another aspect of this embodiment, a BoNT/A comprises SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A comprises a naturally occurring BoNT/A variant, such as, *e.g.*, a BoNT/A isoform or a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A comprises a naturally occurring BoNT/A variant of SEQ ID NO: 1, such as, *e.g.*, a BoNT/A isoform of SEQ ID NO: 1 or a BoNT/A subtype of SEQ ID NO: 1. In still another aspect of this embodiment, a BoNT/A comprises a non-naturally occurring BoNT/A variant, such as, *e.g.*, a conservative BoNT/A variant, a non-conservative BoNT/A variant or an active BoNT/A fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A comprises a non-naturally occurring BoNT/A variant of SEQ ID NO: 1, such as, *e.g.*, a conservative BoNT/A variant of SEQ ID NO: 1, a non-conservative BoNT/A variant of SEQ ID NO: 1 or an active BoNT/A fragment of SEQ ID NO: 1, or any combination thereof. In yet another aspect of this embodiment, a BoNT/A comprises a BoNT/A enzymatic domain or an active fragment thereof, a BoNT/A translocation domain or an active fragment thereof, a BoNT/A binding domain or an active



Steward, L.E. *et al.*, Degradable Clostridial Toxins

fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/A comprising a BoNT/A enzymatic domain of amino acids 1-448 from SEQ ID NO: 1 or an active fragment thereof, a BoNT/A translocation domain of amino acids 449-871 from SEQ ID NO: 1 or an active fragment thereof, a BoNT/A binding domain of amino acids 872-1296 from SEQ ID NO: 1 or an active fragment thereof, and any combination thereof.

**[045]** In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 1, at least 75% amino acid identity with the SEQ ID NO: 1, at least 80% amino acid identity with SEQ ID NO: 1, at least 85% amino acid identity with SEQ ID NO: 1, at least 90% amino acid identity with SEQ ID NO: 1 or at least 95% amino acid identity with SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 1, at most 75% amino acid identity with the SEQ ID NO: 1, at most 80% amino acid identity with SEQ ID NO: 1, at most 85% amino acid identity with SEQ ID NO: 1, at most 90% amino acid identity with SEQ ID NO: 1 or at most 95% amino acid identity with SEQ ID NO: 1.

**[046]** In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 1.

**[047]** In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least

Steward, L.E. *et al.*, Degradable Clostridial Toxins

one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 1.

**[048]** In another embodiment, a Clostridial toxin comprises a BoNT/B. In an aspect of this embodiment, a BoNT/B comprises a BoNT/B enzymatic domain, a BoNT/B translocation domain and a BoNT/B binding domain. In another aspect of this embodiment, a BoNT/B comprises SEQ ID NO: 2. In another aspect of this embodiment, a BoNT/B comprises a naturally occurring BoNT/B variant, such as, *e.g.*, a BoNT/B isoform or a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B comprises a naturally occurring BoNT/B variant of SEQ ID NO: 2, such as, *e.g.*, a BoNT/B isoform of SEQ ID NO: 2 or a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a BoNT/B comprises a non-naturally occurring BoNT/B variant, such as, *e.g.*, a conservative BoNT/B variant, a non-conservative BoNT/B variant or an active BoNT/B fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B comprises a non-naturally occurring BoNT/B variant of SEQ ID NO: 2, such as, *e.g.*, a conservative BoNT/B variant of SEQ ID NO: 2, a non-conservative BoNT/B variant of SEQ ID NO: 2 or an active BoNT/B fragment of SEQ ID NO: 2, or any combination thereof. In yet another aspect of this embodiment, a BoNT/B comprising a BoNT/B enzymatic domain or an active fragment thereof, a BoNT/B translocation domain or active fragment thereof, a BoNT/B binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a BoNT/B comprising a BoNT/B enzymatic domain of amino acids 1-441 from SEQ ID NO: 2 or active fragment thereof, a BoNT/B translocation domain of amino acids 442-858 from SEQ ID NO: 2 or active fragment thereof, a BoNT/B binding domain of amino acids 859-1291 from SEQ ID NO: 2 or active fragment thereof, and any combination thereof.

**[049]** In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 2, at least 75% amino acid identity with the SEQ ID NO: 2, at least 80% amino acid identity with SEQ ID NO: 2, at least 85% amino acid identity with SEQ ID NO: 2, at least 90% amino acid identity with SEQ ID NO: 2 or at least 95% amino acid identity with SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 2, at most 75% amino acid identity with the SEQ ID NO: 2, at most 80% amino acid identity with SEQ ID NO: 2, at most 85% amino acid identity with SEQ ID NO: 2, at most 90% amino acid identity with SEQ ID NO: 2 or at most 95% amino acid identity with SEQ ID NO: 2.

**[050]** In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous

Steward, L.E. *et al.*, Degradable Clostridial Toxins

amino acid substitutions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 2.

[051] In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 2.

[052] In another embodiment, a Clostridial toxin comprises a BoNT/C1. In an aspect of this embodiment, a BoNT/C1 comprises a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain and a BoNT/C1 binding domain. In another aspect of this embodiment, a BoNT/C1 comprises SEQ ID NO: 3. In another aspect of this embodiment, a BoNT/C1 comprises a naturally occurring BoNT/C1 variant, such as, *e.g.*, a BoNT/C1 isoform or a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 comprises a naturally occurring BoNT/C1 variant of SEQ ID NO: 3, such as, *e.g.*, a BoNT/C1 isoform of SEQ ID NO: 3 or a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a BoNT/C1 comprises a non-naturally occurring BoNT/C1 variant, such as, *e.g.*, a conservative BoNT/C1 variant, a non-conservative BoNT/C1 variant or an active BoNT/C1 fragment, or any combination thereof.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

In still another aspect of this embodiment, a BoNT/C1 comprises a non-naturally occurring BoNT/C1 variant of SEQ ID NO: 3, such as, *e.g.*, a conservative BoNT/C1 variant of SEQ ID NO: 3, a non-conservative BoNT/C1 variant of SEQ ID NO: 3 or an active BoNT/C1 fragment of SEQ ID NO: 3, or any combination thereof. In yet another aspect of this embodiment, a BoNT/C1 comprises a BoNT/C1 enzymatic domain or active fragment thereof, a BoNT/C1 translocation domain or active fragment thereof, a BoNT/C1 binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a BoNT/C1 comprises a BoNT/C1 enzymatic domain of amino acid 1-449 from SEQ ID NO: 3 or active fragment thereof, a BoNT/C1 translocation domain of amino acids 450-866 from SEQ ID NO: 3 or active fragment thereof, a BoNT/C1 binding domain of amino acids 867-1291 from SEQ ID NO: 3 or active fragment thereof, and any combination thereof.

**[053]** In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 3, at least 75% amino acid identity with the SEQ ID NO: 3, at least 80% amino acid identity with SEQ ID NO: 3, at least 85% amino acid identity with SEQ ID NO: 3, at least 90% amino acid identity with SEQ ID NO: 3 or at least 95% amino acid identity with SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 3, at most 75% amino acid identity with the SEQ ID NO: 3, at most 80% amino acid identity with SEQ ID NO: 3, at most 85% amino acid identity with SEQ ID NO: 3, at most 90% amino acid identity with SEQ ID NO: 3 or at most 95% amino acid identity with SEQ ID NO: 3.

**[054]** In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 3.

**[055]** In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a

Steward, L.E. *et al.*, Degradable Clostridial Toxins

polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 3.

[056] In another embodiment, a Clostridial toxin comprises a BoNT/D. In an aspect of this embodiment, a BoNT/D comprises a BoNT/D enzymatic domain, a BoNT/D translocation domain and a BoNT/D binding domain. In another aspect of this embodiment, a BoNT/D comprises SEQ ID NO: 4. In another aspect of this embodiment, a BoNT/D comprises a naturally occurring BoNT/D variant, such as, *e.g.*, a BoNT/D isoform or a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D comprises a naturally occurring BoNT/D variant of SEQ ID NO: 4, such as, *e.g.*, a BoNT/D isoform of SEQ ID NO: 4 or a BoNT/D subtype of SEQ ID NO: 4. In still another aspect of this embodiment, a BoNT/D comprises a non-naturally occurring BoNT/D variant, such as, *e.g.*, a conservative BoNT/D variant, a non-conservative BoNT/D variant or an active BoNT/D fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D comprises a non-naturally occurring BoNT/D variant of SEQ ID NO: 4, such as, *e.g.*, a conservative BoNT/D variant of SEQ ID NO: 4, a non-conservative BoNT/D variant of SEQ ID NO: 4 or an active BoNT/D fragment of SEQ ID NO: 4, or any combination thereof. In yet another aspect of this embodiment, a BoNT/D comprises a BoNT/D enzymatic domain or an active fragment thereof, a BoNT/D translocation domain or an active fragment thereof, a BoNT/D binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/D comprising a BoNT/D enzymatic domain of amino acids 1-445 from SEQ ID NO: 4 or an active fragment thereof, a BoNT/D translocation domain of amino acids 446-862 from SEQ ID NO: 4 or an active fragment thereof, a BoNT/D binding domain of amino acids 863-1276 from SEQ ID NO: 4 or an active fragment thereof, and any combination thereof.

[057] In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 4, at least 75% amino acid identity with the SEQ ID NO: 4, at least 80% amino acid identity with SEQ ID NO: 4, at least 85% amino acid identity with SEQ ID NO: 4, at least 90% amino acid identity with SEQ ID NO: 4 or at least 95% amino acid identity with SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 4, at most 75% amino acid identity with the SEQ ID NO: 4, at most

Steward, L.E. *et al.*, Degradable Clostridial Toxins

80% amino acid identity with SEQ ID NO: 4, at most 85% amino acid identity with SEQ ID NO: 4, at most 90% amino acid identity with SEQ ID NO: 4 or at most 95% amino acid identity with SEQ ID NO: 4.

[058] In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 4.

[059] In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 4.

[060] In another embodiment, a Clostridial toxin comprises a BoNT/E. In an aspect of this embodiment, a BoNT/E comprises a BoNT/E enzymatic domain, a BoNT/E translocation domain and a BoNT/E binding domain. In another aspect of this embodiment, a BoNT/E comprises SEQ ID NO: 5. In another aspect of this embodiment, a BoNT/E comprises a naturally occurring BoNT/E variant, such as, *e.g.*, a BoNT/E

Steward, L.E. *et al.*, Degradable Clostridial Toxins

isoform or a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E comprises a naturally occurring BoNT/E variant of SEQ ID NO: 5, such as, *e.g.*, a BoNT/E isoform of SEQ ID NO: 5 or a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a BoNT/E comprises a non-naturally occurring BoNT/E variant, such as, *e.g.*, a conservative BoNT/E variant, a non-conservative BoNT/E variant or an active BoNT/E fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E comprises a non-naturally occurring BoNT/E variant of SEQ ID NO: 5, such as, *e.g.*, a conservative BoNT/E variant of SEQ ID NO: 5, a non-conservative BoNT/E variant of SEQ ID NO: 5 or an active BoNT/E fragment of SEQ ID NO: 5, or any combination thereof. In yet another aspect of this embodiment, a BoNT/E comprising a BoNT/E enzymatic domain or an active fragment thereof, a BoNT/E translocation domain or active fragment thereof, a BoNT/E binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a BoNT/E comprising a BoNT/E enzymatic domain of amino acids 1-422 from SEQ ID NO: 5 or active fragment thereof, a BoNT/E translocation domain of amino acids 423-845 from SEQ ID NO: 5 or active fragment thereof, a BoNT/E binding domain of amino acids 846-1252 from SEQ ID NO: 5 or active fragment thereof, and any combination thereof.

**[061]** In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 5, at least 75% amino acid identity with the SEQ ID NO: 5, at least 80% amino acid identity with SEQ ID NO: 5, at least 85% amino acid identity with SEQ ID NO: 5, at least 90% amino acid identity with SEQ ID NO: 5 or at least 95% amino acid identity with SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 5, at most 75% amino acid identity with the SEQ ID NO: 5, at most 80% amino acid identity with SEQ ID NO: 5, at most 85% amino acid identity with SEQ ID NO: 5, at most 90% amino acid identity with SEQ ID NO: 5 or at most 95% amino acid identity with SEQ ID NO: 5.

**[062]** In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two,

Steward, L.E. *et al.*, Degradable Clostridial Toxins

three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 5.

**[063]** In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40 , 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 5.

**[064]** In another embodiment, a Clostridial toxin comprises a BoNT/F. In an aspect of this embodiment, a BoNT/F comprises a BoNT/F enzymatic domain, a BoNT/F translocation domain and a BoNT/F binding domain. In another aspect of this embodiment, a BoNT/F comprises SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/F comprises a naturally occurring BoNT/F variant, such as, *e.g.*, a BoNT/F isoform or a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F comprises a naturally occurring BoNT/F variant of SEQ ID NO: 6, such as, *e.g.*, a BoNT/F isoform of SEQ ID NO: 6 or a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a BoNT/F comprises a non-naturally occurring BoNT/F variant, such as, *e.g.*, a conservative BoNT/F variant, a non-conservative BoNT/F variant or an active BoNT/F fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F comprises a non-naturally occurring BoNT/F variant of SEQ ID NO: 6, such as, *e.g.*, a conservative BoNT/F variant of SEQ ID NO: 6, a non-conservative BoNT/F variant of SEQ ID NO: 6 or an active BoNT/F fragment of SEQ ID NO: 6, or any combination thereof. In yet another aspect of this embodiment, a BoNT/F comprises a BoNT/F enzymatic domain or active fragment thereof, a BoNT/F translocation domain or active fragment thereof, a BoNT/F binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a BoNT/F comprises a BoNT/F enzymatic domain of amino acid 1-439 from SEQ ID NO: 6 or active fragment thereof, a BoNT/F translocation domain of amino acids 440-864 from SEQ ID NO: 6 or active fragment thereof, a BoNT/F binding domain of amino acids 865-1274 from SEQ ID NO: 6 or active fragment thereof, and any combination thereof.



Steward, L.E. *et al.*, Degradable Clostridial Toxins

**[065]** In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 6, at least 75% amino acid identity with the SEQ ID NO: 6, at least 80% amino acid identity with SEQ ID NO: 6, at least 85% amino acid identity with SEQ ID NO: 6, at least 90% amino acid identity with SEQ ID NO: 6 or at least 95% amino acid identity with SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 6, at most 75% amino acid identity with the SEQ ID NO: 6, at most 80% amino acid identity with SEQ ID NO: 6, at most 85% amino acid identity with SEQ ID NO: 6, at most 90% amino acid identity with SEQ ID NO: 6 or at most 95% amino acid identity with SEQ ID NO: 6.

**[066]** In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 6.

**[067]** In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six,

Steward, L.E. *et al.*, Degradable Clostridial Toxins

seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 6.

**[068]** In another embodiment, a Clostridial toxin comprises a BoNT/G. In an aspect of this embodiment, a BoNT/G comprises a BoNT/G enzymatic domain, a BoNT/G translocation domain and a BoNT/G binding domain. In another aspect of this embodiment, a BoNT/G comprises SEQ ID NO: 7. In another aspect of this embodiment, a BoNT/G comprises a naturally occurring BoNT/G variant, such as, *e.g.*, a BoNT/G isoform or a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G comprises a naturally occurring BoNT/G variant of SEQ ID NO: 7, such as, *e.g.*, a BoNT/G isoform of SEQ ID NO: 7 or a BoNT/G subtype of SEQ ID NO: 7. In still another aspect of this embodiment, a BoNT/G comprises a non-naturally occurring BoNT/G variant, such as, *e.g.*, a conservative BoNT/G variant, a non-conservative BoNT/G variant or an active BoNT/G fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D comprises a non-naturally occurring BoNT/G variant of SEQ ID NO: 7, such as, *e.g.*, a conservative BoNT/G variant of SEQ ID NO: 7, a non-conservative BoNT/G variant of SEQ ID NO: 7 or an active BoNT/G fragment of SEQ ID NO: 7, or any combination thereof. In yet another aspect of this embodiment, a BoNT/G comprises a BoNT/G enzymatic domain or an active fragment thereof, a BoNT/G translocation domain or an active fragment thereof, a BoNT/G binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/G comprising a BoNT/G enzymatic domain of amino acids 1-446 from SEQ ID NO: 7 or an active fragment thereof, a BoNT/G translocation domain of amino acids 447-863 from SEQ ID NO: 7 or an active fragment thereof, a BoNT/G binding domain of amino acids 864-1297 from SEQ ID NO: 7 or an active fragment thereof, and any combination thereof.

**[069]** In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 7, at least 75% amino acid identity with the SEQ ID NO: 7, at least 80% amino acid identity with SEQ ID NO: 7, at least 85% amino acid identity with SEQ ID NO: 7, at least 90% amino acid identity with SEQ ID NO: 7 or at least 95% amino acid identity with SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 7, at most 75% amino acid identity with the SEQ ID NO: 7, at most 80% amino acid identity with SEQ ID NO: 7, at most 85% amino acid identity with SEQ ID NO: 7, at most 90% amino acid identity with SEQ ID NO: 7 or at most 95% amino acid identity with SEQ ID NO: 7.

**[070]** In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions

Steward, L.E. *et al.*, Degradable Clostridial Toxins

relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 7.

**[071]** In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 7.

**[072]** In another embodiment, a Clostridial toxin comprises a TeNT. In an aspect of this embodiment, a TeNT comprises a TeNT enzymatic domain, a TeNT translocation domain and a TeNT binding domain. In an aspect of this embodiment, a TeNT comprises SEQ ID NO: 8. In another aspect of this embodiment, a TeNT comprises a naturally occurring TeNT variant, such as, *e.g.*, a TeNT isoform or a TeNT subtype. In another aspect of this embodiment, a TeNT comprises a naturally occurring TeNT variant of SEQ ID NO: 8, such as, *e.g.*, a TeNT isoform of SEQ ID NO: 8 or a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a TeNT comprises a non-naturally occurring TeNT variant, such as, *e.g.*, a conservative TeNT variant, a non-conservative TeNT variant or an active TeNT fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT comprises a non-naturally occurring TeNT variant of SEQ ID NO: 8, such as, *e.g.*, a conservative TeNT variant of SEQ ID NO: 8, a non-conservative TeNT variant of SEQ ID NO: 8 or an active TeNT fragment of SEQ ID NO: 8, or any combination thereof. In yet another aspect of this embodiment, a TeNT comprising a TeNT enzymatic domain or an active fragment thereof, a TeNT translocation domain or active fragment thereof, a TeNT binding domain or active fragment thereof, and any combination thereof. In yet another aspect of

Steward, L.E. *et al.*, Degradable Clostridial Toxins

this embodiment, a TeNT comprising a TeNT enzymatic domain of amino acids 1-457 from SEQ ID NO: 8 or active fragment thereof, a TeNT translocation domain of amino acids 458-879 from SEQ ID NO: 8 or active fragment thereof, a TeNT binding domain of amino acids 880-1315 from SEQ ID NO: 8 or active fragment thereof, and any combination thereof.

**[073]** In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 8, at least 75% amino acid identity with the SEQ ID NO: 8, at least 80% amino acid identity with SEQ ID NO: 8, at least 85% amino acid identity with SEQ ID NO: 8, at least 90% amino acid identity with SEQ ID NO: 8 or at least 95% amino acid identity with SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 8, at most 75% amino acid identity with the SEQ ID NO: 8, at most 80% amino acid identity with SEQ ID NO: 8, at most 85% amino acid identity with SEQ ID NO: 8, at most 90% amino acid identity with SEQ ID NO: 8 or at most 95% amino acid identity with SEQ ID NO: 8.

**[074]** In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 8.

**[075]** In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid

Steward, L.E. *et al.*, Degradable Clostridial Toxins

deletions relative to SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 8.

**[076]** Aspects of the present invention provide, in part, a PAR ligand domain. As used herein, the term "PAR ligand domain" is synonymous with "tethered ligand" and "activating peptide" and means any polypeptide that can selectively bind to the PAR ligand binding domain and initiate the overall internalization mechanism whereby an activated PAR is internalized into a cell. As used herein, the term "selectively" means having a unique effect or influence or reacting in only one way or with only one thing. As used herein, the term "selectively bind" means that a PAR ligand domain is able to bind its target PAR ligand binding domain under physiological conditions, or in vitro conditions substantially approximating physiological conditions, to a statistically significantly greater degree (*i.e.*, has a smaller  $K_d$  or dissociation constant) than to other, non-target ligand binding domains. " $K_d$ " is the molar concentration of the PAR ligand domain at which half the PAR ligand binding domains are bound by the PAR ligand domain. Thus, there is a discriminatory binding of the PAR ligand domain to the indicated target binding site.

**[077]** Most G protein-coupled receptors (GPCRs) are reversibly activated upon ligand binding. However, activation of protease-activated G protein-coupled receptors (PARs) occurs through an irreversible proteolytic event that results in the generation of a tethered ligand that cannot diffuse away. In essence, PARs are receptors that carry their own ligands, which remain unbound until unmasked by site-specific receptor cleavage. The coagulant protease Thrombin is the physiological activator of PAR1, PAR3 and PAR4; however, other proteases can cleave these receptors and may contribute to their function in vivo (Table 2). PAR2 is activated by multiple Trypsin-like serine proteases including Trypsin, Tryptase and coagulation proteases upstream of Thrombin, Factors VIIa and Xa, but not by Thrombin (Table 2).

**[078]** Currently four subtypes of human PARs are described and designated PAR1 (SEQ ID NO: 9), PAR2 (SEQ ID NO: 10), PAR3 (SEQ ID NO: 11) and PAR4 (SEQ ID NO: 12). In addition, PAR1, PAR2, PAR3 and PAR4 orthologs which exhibit at least 70% amino acid identity and at least 80% amino acid similarity have been identified in other mammals, such as, *e.g.*, the chimpanzee *Pan troglodytes*, the hamadryas baboon *Papio hamadryas*, the dog *Canis familiaris*, the mouse *Mus musculus*, the rat *Rattus norvegicus* and the chicken *Gallus gallus*. The protease cleavage site, which upon cleavage unmasks the tethered ligand, is known for all four receptors (Table 2). In human PARs, cleavage of PAR1 at R41-S42 exposes a new amino terminus ending in the hexapeptide SFLLRN, cleavage of PAR2 at R34-S35 exposes a new amino terminus ending in the hexapeptide SLIGKV, cleavage of PAR3 at K38-T39 exposes a new amino terminus ending in the hexapeptide TFRGAP, where as, cleavage of PAR4 at R47-G48 exposes a new amino terminus ending in the hexapeptide GYPGQV. A hirudin-like site distal to the

Steward, L.E. *et al.*, Degradable Clostridial Toxins

protease cleavage site has been described in PAR1 and PAR3. This charged domain appears to help mediate the binding of Thrombin to PAR1, thereby facilitating cleavage of the protease cleavage site.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Table 2 Summary of the Human PAR Family				
	PAR1	PAR2	PAR3	PAR4
Endogenous Activating proteases	APC Factor Xa Thrombin Trypsins	Acrosien Factor Xa Factor VIIa MT-SP1 Proteinase-3 Trypsins Trypsins	Thrombin	Cathepsin G Factor Xa Factor VIIa Plasmin Thrombin Trypsins
Exogenous Activating proteases	Granzyme A Gingipains-R	Der P1 Der P3 Der P9 Gingipains-R		Gingipains-R
Inactivating proteases	Cathepsin G Elastase Plasmin Proteinase-3 Trypsins	Cathepsin G Elastase	Cathepsin G	
Cleavage site	LDPR <sup>41</sup> *S <sup>42</sup> FLLRN	SKGR <sup>34</sup> *S <sup>35</sup> LIGKV	LPIK <sup>38</sup> *T <sup>39</sup> FRGAP	PAPR <sup>47</sup> *G <sup>48</sup> YPGQV
Localization	platelets endothelium epithelium fibroblasts myocytes neurons astrocytes	epithelium endothelium fibroblasts myocytes neurons astrocytes	platelets endothelium myocytes astrocytes	platelets endothelium myocytes astrocytes
An asterisks (*) indicates the peptide bond that is cleaved by an activating PAR protease.				

[079] Synthetic peptides representing the newly formed amino terminus tethered ligand of PARs can act as agonists for the receptor without the need for proteolysis and can initiate many of the same signaling responses elicited by proteolytically activated PARs (Table 3), see *e.g.*, Shaun R. Coughlin and Mark Kahn, *Modulation of Platelet Activation*, PCT Patent Publication WO 01/07072 (Feb. 1, 2001); Shaun R. Coughlin and Tatjana R. Faruqi, *Peptides Modulating Protease Activated Receptors and Methods of Using Same*, PCT Patent Publication WO 01/94411 (Dec. 13, 2001); Scott R. MacFarlane *et al.*, *Protease-Activated Receptors*, 53(2) Pharmacol. Rev. 245-282 (2001); and Robert M. Scarborough, *Protease-Activated Receptor-2 Antagonists and Agonists*, 1(1) Curr. Med. Chem. Cardiovasc. Hematol. Agents 73-82 (2003). Referred to as activating peptides (AP), these peptides evoke the ligand binding, the signal transduction and the signal termination steps described above. The first described AP was the 14-residue peptide SFLLRNPNDKYEPF comprising amino acids 42-55 of SEQ ID NO: 13 that behaves as an agonist for PAR1. Subsequent work has shown that not only the hexapeptide SFLLRN, but a wide range of variants were also effective, if not fully functional to elicit a cellular response (Table 3). Analysis of PAR APs using alanine scanning and site-directed mutagenesis has identified residues critical for function. For example, the residues F2, L4 and R5 are functionally important for the PAR1 AP hexapeptide SFLLRN, but substitutions of residues at other positions can be tolerated. Similar testing of the PAR2 AP hexapeptide SLIGKV indicates that L2 and R5 are essential for PAR2 AP activity whereas substitution of G4 or L6 has only a slight effect on PAR2 activation. Replacing S1 or I3 with alanine also

Steward, L.E. *et al.*, Degradable Clostridial Toxins

reduces activity. While many PAR4 variants are functional (Table 3), the specificity of PAR4 AP requires Y2, since replacement with F generates an agonist of both PAR1 and PAR4.

Table 3. PAR Binding Domains		
PAR1	Amino Acid Sequence	SEQ ID NO:
Reference	SFLLRN	13
Variants	SFFLRN	14
	SFFLKN	133
	TFLLRN	15
	GFPGKF	16
	GYPAKF	17
	GYPLKF	18
	GYPEKF	19
	G(F)PGKF	20
	GYP(Cha)KF	21
	S(F)(Cha)(Cha)RK	22
	S(F)(Cha)(Cha)(homoR)K	23
PAR2	Amino Acid Sequence	SEQ ID NO:
Reference	SLIGKV	24
Variants	SLIGRL	25
PAR3	Amino Acid Sequence	SEQ ID NO:
Reference	TFRGAP	26
Variants	SFNGGP	27
	SFNGNE	134
PAR4	Amino Acid Sequence	SEQ ID NO:
Reference	GYPGQV	28
Variants	AYPGKF	29
	TYPGKF	30
	GYPGKY	31
	GYPGKW	32
	GYPGKK	33
	GYPGKF	34
	GYPGRF	35
	GYPGFK	36
	GYPAKF	37
	GFPGKF	38
	GFPGKP	39
	SYPGKF	40
	SYPAKF	41
	SYPGRF	42
	SYAGKF	43
	SFPGQP	135
	SFPGQA	160
	GYPG(Orn)F	44
	G(F)PGKF	45
	GYPG(homoR)F	46
	SYPG(homoR)F	47
(Cha), cyclohexylalanine; (homoR), homoarginine; (Orn), ornithine; (F), parafluoro-phenylalanine; other letters represent the single letter amino acid code.		



Steward, L.E. *et al.*, Degradable Clostridial Toxins

**[080]** It is envisioned that any and all PAR ligand domains capable of binding an inactivated PAR and eliciting the internalization of the modified Clostridial toxin-PAR complex into a cell can be useful in aspects of the present invention. It is envisioned that a PAR ligand domain of any and all lengths can be useful in aspects of the present invention with the proviso that the PAR ligand domain is capable of binding an inactivated PAR and eliciting the internalization of the modified Clostridial toxin-PAR complex into a cell. Thus, in aspects of this embodiment, a PAR ligand domain can be, *e.g.*, at least 6 amino acids in length, at least 7 amino acids in length, at least 8 amino acids in length, at least 9 amino acids in length, at least 10 amino acids in length, at least 15 amino acids in length, at least 20 amino acids in length, at least 25 amino acids in length, at least 30 amino acids in length, at least 40 amino acids in length, at least 50 amino acids in length or at least 60 amino acids in length. In other aspects of this embodiment, a PAR ligand domain can be, *e.g.*, at most 6 amino acids in length, at most 7 amino acids in length, at most 8 amino acids in length, at most 9 amino acids in length, at most 10 amino acids in length, at most 15 amino acids in length, at most 20 amino acids in length, at most 25 amino acids in length, at most 30 amino acids in length, at most 40 amino acids in length, at most 50 amino acids in length or at most 60 amino acids in length. As a non-limiting example, a PAR 1 ligand domain can comprise amino acids 1-64 of SEQ ID NO: 9, amino acids 1-55 of SEQ ID NO: 9, amino acids 1-47 of SEQ ID NO: 9, amino acids 29-64 of SEQ ID NO: 9, amino acids 42-55 of SEQ ID NO: 9 or amino acids 42-47 of SEQ ID NO: 9. As another non-limiting example, a PAR 2 ligand domain can comprise amino acids 1-59 of SEQ ID NO: 10, comprise amino acids 1-48 of SEQ ID NO: 10, comprise amino acids 1-40 of SEQ ID NO: 10, amino acids 24-59 of SEQ ID NO: 10, amino acids 35-48 of SEQ ID NO: 10 or amino acids 35-40 of SEQ ID NO: 10. As still another non-limiting example, a PAR 3 ligand domain can comprise amino acids 1-60 of SEQ ID NO: 11, comprise amino acids 1-52 of SEQ ID NO: 11, comprise amino acids 1-44 of SEQ ID NO: 11, amino acids 26-60 of SEQ ID NO: 11, amino acids 39-52 of SEQ ID NO: 11 or amino acids 39-44 of SEQ ID NO: 11. As yet another non-limiting example, a PAR 4 ligand domain can comprise amino acids 1-70 of SEQ ID NO: 12, comprise amino acids 1-61 of SEQ ID NO: 12, comprise amino acids 1-53 of SEQ ID NO: 12, amino acids 35-70 of SEQ ID NO: 12, amino acids 48-61 of SEQ ID NO: 12 or amino acids 48-53 of SEQ ID NO: 12.

**[081]** A PAR ligand domain useful in aspects of the invention includes, without limitation, naturally occurring PAR ligand domains, such as, *e.g.*, a PAR1 tethered ligand, a PAR2 tethered ligand, a PAR3 tethered ligand or a PAR4 tethered ligand; naturally occurring PAR ligand domain variants; and non-naturally-occurring PAR ligand domain variants, such as, *e.g.*, conservative PAR ligand domain variants, non-conservative PAR ligand domain variants and PAR ligand domain peptidomimetics. As used herein, the term "PAR ligand domain variant," whether naturally-occurring or non-naturally-occurring, means a PAR ligand domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences and can be described in percent identity to the corresponding region of that reference sequence (Table 3). Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid

Steward, L.E. *et al.*, Degradable Clostridial Toxins

methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

**[082]** As used herein, the term “naturally occurring PAR ligand domain variant” means any PAR ligand domain produced without the aid of any human manipulation, including, without limitation, PAR ligand domain isoforms produced from alternatively-spliced transcripts, PAR ligand domain isoforms produced by spontaneous mutation and PAR ligand domain subtypes.

**[083]** As used herein, the term “non-naturally occurring PAR ligand domain variant” means any PAR ligand domain produced with the aid of human manipulation, including, without limitation, PAR ligand domain variants produced by genetic engineering using random mutagenesis or rational design and PAR ligand domain variants produced by chemical synthesis. Non-limiting examples of non-naturally occurring PAR ligand domain variant include, *e.g.*, conservative PAR ligand domain variants, non-conservative PAR ligand domain variants and PAR ligand domain peptidomimetics.

**[084]** As used herein, the term “conservative PAR ligand domain variant” means a PAR ligand domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference PAR ligand domain sequence (Table 3). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative PAR ligand domain variant can function in substantially the same manner as the reference PAR ligand domain on which the conservative PAR ligand domain variant is based, and can be substituted for the reference PAR ligand domain in any aspect of the present invention. A conservative PAR ligand domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids or five or more amino acids from the reference PAR ligand domain on which the conservative PAR ligand domain variant is based. A conservative PAR ligand domain variant can also possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference PAR ligand domain on which the conservative PAR ligand domain variant is based. Non-limiting examples of a conservative PAR ligand domain variant include, *e.g.*, conservative PAR1 ligand domain variants, conservative PAR2 ligand domain variants, conservative PAR3 ligand domain variants and conservative PAR4 ligand domain variants.

**[085]** As used herein, the term “non-conservative PAR ligand domain variant” means a PAR ligand domain in which 1) at least one amino acid is deleted from the reference PAR ligand domain on which the non-conservative PAR ligand domain variant is based; 2) at least one amino acid added to the reference PAR ligand domain on which the non-conservative PAR ligand domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference PAR ligand domain sequence (Table 3). A

Steward, L.E. *et al.*, Degradable Clostridial Toxins

non-conservative PAR ligand domain variant can function in substantially the same manner as the reference PAR ligand domain on which the non-conservative PAR ligand domain is based, and can be substituted for the reference PAR ligand domain in any aspect of the present invention. A non-conservative PAR ligand domain variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference PAR ligand domain on which the non-conservative PAR ligand domain variant is based. A non-conservative PAR ligand domain may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids or five or more amino acids from the reference PAR ligand domain on which the non-conservative PAR ligand domain variant is based. A non-conservative PAR ligand domain variant can also possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference PAR ligand domain on which the non-conservative PAR ligand domain variant is based. Non-limiting examples of a non-conservative PAR ligand domain variant include, *e.g.*, non-conservative PAR1 ligand domain variants, non-conservative PAR2 ligand domain variants, non-conservative PAR3 ligand domain variants and non-conservative PAR4 ligand domain variants.

**[086]** As used herein, the term "PAR ligand domain peptidomimetic" means a PAR ligand domain that has at least one amino acid substituted by a non-natural oligomer that has at least one property similar to that of the first amino acid. Examples of properties include, without limitation, topography of a peptide primary structural element, functionality of a peptide primary structural element, topology of a peptide secondary structural element, functionality of a peptide secondary structural element, of the like, or any combination thereof. A PAR ligand domain peptidomimetic can function in substantially the same manner as the reference PAR ligand domain on which the PAR ligand domain peptidomimetic is based, and can be substituted for the reference PAR ligand domain in any aspect of the present invention. A PAR ligand domain peptidomimetic may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids or five or more amino acids from the reference PAR ligand domain on which the PAR ligand domain peptidomimetic is based. A PAR ligand domain peptidomimetic can also possess at least 50% amino acid identity, at least 65% amino acid identity, at least 75% amino acid identity, at least 85% amino acid identity or at least 95% amino acid identity to the reference PAR ligand domain on which the PAR ligand domain peptidomimetic is based. For examples of peptidomimetic methods see, *e.g.*, Amy S. Ripka & Daniel H. Rich, Peptidomimetic design, 2(4) CURR. OPIN. CHEM. BIOL. 441-452 (1998); and M. Angels Estiarte & Daniel H. Rich, *Peptidomimetics for Drug Design*, 803-861 (BURGER'S MEDICINAL CHEMISTRY AND DRUG DISCOVERY Vol. 1 PRINCIPLE AND PRACTICE, Donald J. Abraham ed., Wiley-Interscience, 6<sup>th</sup> ed 2003). Non-limiting examples of a PAR ligand domain peptidomimetic include, *e.g.*, PAR1 ligand domain peptidomimetics, PAR2 ligand domain peptidomimetics, PAR3 ligand domain peptidomimetics and PAR4 ligand domain peptidomimetics.

**[087]** Thus, in an embodiment, a PAR ligand domain comprises a naturally occurring PAR ligand domain variant, such as, *e.g.*, a PAR ligand domain isoform or a PAR ligand domain subtype. In another

Steward, L.E. *et al.*, Degradable Clostridial Toxins

embodiment a PAR ligand domain comprises a non-naturally occurring PAR ligand domain variant, such as, *e.g.*, a conservative PAR ligand domain variant, a non-conservative PAR ligand domain variant or a PAR ligand domain peptidomimetic, or any combination thereof.

**[088]** In another embodiment, a PAR ligand domain comprises a PAR1 ligand domain. In an aspect of this embodiment, a PAR1 ligand domain comprises SEQ ID NO: 13. In another aspect of this embodiment, a PAR1 ligand domain comprises a naturally occurring PAR1 ligand domain variant, such as, *e.g.*, a PAR1 ligand domain isoform or a PAR1 ligand domain subtype. In another aspect of this embodiment, a PAR1 ligand domain comprises a naturally occurring PAR1 ligand domain variant of SEQ ID NO: 13, such as, *e.g.*, a PAR1 ligand domain isoform of SEQ ID NO: 13 or a PAR1 ligand domain subtype of SEQ ID NO: 13. In still another aspect of this embodiment, a PAR1 ligand domain comprises a non-naturally occurring PAR1 ligand domain variant, such as, *e.g.*, a conservative PAR1 ligand domain variant, a non-conservative PAR1 ligand domain variant or a PAR1 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a PAR1 ligand domain comprises a non-naturally occurring PAR1 ligand domain variant of SEQ ID NO: 13, such as, *e.g.*, a conservative PAR1 ligand domain variant of SEQ ID NO: 13, a non-conservative PAR1 ligand domain variant of SEQ ID NO: 13 or a PAR1 ligand domain peptidomimetic of SEQ ID NO: 13, or any combination thereof. In other aspects of this embodiment, a PAR1 ligand domain comprises SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 or SEQ ID NO: 133.

**[089]** In other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 13, at least 67% amino acid identity with the SEQ ID NO: 13, or at least 83% amino acid identity with SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 13, at most 67% amino acid identity with the SEQ ID NO: 13, at most 83% amino acid identity with SEQ ID NO: 13.

**[090]** In other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ

ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 13.

[091] In other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 13.

[092] In another embodiment, a PAR ligand domain comprises a PAR2 ligand domain. In an aspect of this embodiment, a PAR2 ligand domain comprises SEQ ID NO: 24. In another aspect of this embodiment, a PAR2 ligand domain comprises a naturally occurring PAR2 ligand domain variant, such as, *e.g.*, a PAR2 ligand domain isoform or a PAR2 ligand domain subtype. In another aspect of this embodiment, a PAR2 ligand domain comprises a naturally occurring PAR2 ligand domain variant of SEQ ID NO: 24, such as, *e.g.*, a PAR2 ligand domain isoform of SEQ ID NO: 24 or a PAR2 ligand domain subtype of SEQ ID NO: 24. In still another aspect of this embodiment, a PAR2 ligand domain comprises a non-naturally occurring PAR2 ligand domain variant, such as, *e.g.*, a conservative PAR2 ligand domain variant, a non-conservative PAR2 ligand domain variant or a PAR2 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a PAR2 ligand domain comprises a non-naturally occurring PAR2 ligand domain variant of SEQ ID NO: 24, such as, *e.g.*, a conservative PAR2 ligand domain variant of SEQ ID NO: 24, a non-conservative PAR2 ligand domain variant of SEQ ID NO: 24 or a PAR2 ligand domain peptidomimetic of SEQ ID NO: 24, or any combination thereof. In other aspects of this embodiment, a PAR2 ligand domain comprises SEQ ID NO: 24 or SEQ ID NO: 25.

[093] In other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 24, at least 67% amino acid identity with the SEQ ID NO: 24, or at least 83% amino acid identity with SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 24, at most 67% amino acid identity with the SEQ ID NO: 24, at most 83% amino acid identity with SEQ ID NO: 24.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

**[094]** In other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 24.

**[095]** In other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 24.

**[096]** In another embodiment, a PAR ligand domain comprises a PAR3 ligand domain. In an aspect of this embodiment, a PAR3 ligand domain comprises SEQ ID NO: 26. In another aspect of this embodiment, a PAR3 ligand domain comprises a naturally occurring PAR3 ligand domain variant, such as, *e.g.*, a PAR3 ligand domain isoform or a PAR3 ligand domain subtype. In another aspect of this embodiment, a PAR3 ligand domain comprises a naturally occurring PAR3 ligand domain variant of SEQ ID NO: 26, such as, *e.g.*, a PAR3 ligand domain isoform of SEQ ID NO: 26 or a PAR3 ligand domain subtype of SEQ ID NO: 26. In still another aspect of this embodiment, a PAR3 ligand domain comprises a non-naturally occurring PAR3 ligand domain variant, such as, *e.g.*, a conservative PAR3 ligand domain variant, a non-conservative PAR3 ligand domain variant or a PAR3 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a PAR3 ligand domain comprises a non-naturally occurring PAR3 ligand domain variant of SEQ ID NO: 26, such as, *e.g.*, a conservative PAR3 ligand domain variant of SEQ ID NO: 26, a non-conservative PAR3 ligand domain variant of SEQ ID NO: 26 or a PAR3 ligand domain peptidomimetic of SEQ ID NO: 26, or any combination thereof. In other

Steward, L.E. *et al.*, Degradable Clostridial Toxins

aspects of this embodiment, a PAR3 ligand domain comprises SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 134.

[097] In other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 26, at least 67% amino acid identity with the SEQ ID NO: 26, or at least 83% amino acid identity with SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 26, at most 67% amino acid identity with the SEQ ID NO: 26, at most 83% amino acid identity with SEQ ID NO: 26.

[098] In other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 26.

[099] In other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 26.

[0100] In another embodiment, a PAR ligand domain comprises a PAR4 ligand domain. In an aspect of this embodiment, a PAR4 ligand domain comprises SEQ ID NO: 28. In another aspect of this embodiment, a PAR4 ligand domain comprises a naturally occurring PAR4 ligand domain variant, such

Steward, L.E. *et al.*, Degradable Clostridial Toxins

as, *e.g.*, a PAR4 ligand domain isoform or a PAR4 ligand domain subtype. In another aspect of this embodiment, a PAR4 ligand domain comprises a naturally occurring PAR4 ligand domain variant of SEQ ID NO: 28, such as, *e.g.*, a PAR4 ligand domain isoform of SEQ ID NO: 28 or a PAR4 ligand domain subtype of SEQ ID NO: 28. In still another aspect of this embodiment, a PAR4 ligand domain comprises a non-naturally occurring PAR4 ligand domain variant, such as, *e.g.*, a conservative PAR4 ligand domain variant, a non-conservative PAR4 ligand domain variant or a PAR4 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a PAR4 ligand domain comprises a non-naturally occurring PAR4 ligand domain variant of SEQ ID NO: 28, such as, *e.g.*, a conservative PAR4 ligand domain variant of SEQ ID NO: 28, a non-conservative PAR4 ligand domain variant of SEQ ID NO: 28 or a PAR4 ligand domain peptidomimetic of SEQ ID NO: 28, or any combination thereof. In other aspects of this embodiment, a PAR4 ligand domain comprises SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 135 or SEQ ID NO: 160.

**[0101]** In other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 28, at least 67% amino acid identity with the SEQ ID NO: 28, or at least 83% amino acid identity with SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 28, at most 67% amino acid identity with the SEQ ID NO: 28, at most 83% amino acid identity with SEQ ID NO: 28.

**[0102]** In other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 28.

**[0103]** In other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least two,



Steward, L.E. *et al.*, Degradable Clostridial Toxins

three or four contiguous amino acid substitutions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 28.

**[0104]** When a PAR protease cleaves the extracellular amino-terminus of a PAR, a new amino acid terminus is generated that functions as a tethered ligand. Currently it is believed that the amino terminus location of the tethered ligand is critical for the ligand to effectively bind to the second extracellular loop region of the receptor that comprises the ligand binding domain. It is envisioned that a modified Clostridial toxin of the present specification can comprise a PAR ligand domain in any and all locations with the proviso that formation of the di-chain molecule will result in the free amino terminus of the PAR ligand domain. Non-limiting examples include, locating the PAR ligand domain at the amino terminus of the Clostridial toxin enzymatic domain; locating the PAR ligand domain at the amino terminus of the Clostridial toxin translocation domain; and locating the PAR ligand domain at the amino terminus of the Clostridial toxin binding domain (FIG. 4).

**[0105]** Thus, in an embodiment, a modified Clostridial toxin comprises a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin enzymatic domain. In an aspect of this embodiment, the PAR ligand domain can be located at the amino terminus of the enzymatic domain when the amino to carboxyl linear organization of the Clostridial toxin single chain molecule is enzymatic domain, translocation domain and binding domain. In another aspect of this embodiment, the PAR ligand domain can be located at the amino terminus of the enzymatic domain when the amino to carboxyl linear organization of the Clostridial toxin single chain molecule is enzymatic domain, binding domain and translocation domain.

**[0106]** In another embodiment, a modified Clostridial toxin comprises a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin translocation domain. In an aspect of this embodiment, the PAR ligand domain can also be located at the amino terminus of the translocation domain when the amino to carboxyl linear organization of the Clostridial toxin single chain molecule is binding domain, enzymatic domain and translocation domain. In another aspect of this embodiment, the PAR ligand domain can also be located at the amino terminus of the translocation domain when the amino to carboxyl linear organization of the Clostridial toxin single chain

Steward, L.E. *et al.*, Degradable Clostridial Toxins

molecule is enzymatic domain, translocation domain and binding domain.

[0107] In still another embodiment, a modified Clostridial toxin comprises a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin binding domain. In an aspect of this embodiment, the PAR ligand domain can also be located at the amino terminus of the binding domain when the amino to carboxyl linear organization of the Clostridial toxin single chain molecule is enzymatic domain, binding domain and translocation domain.

Table 4: Amino Terminus Region			
Toxin	SEQ ID NO	PAR Ligand Domain	Light Chain Region
BoNT/A	1	M-PAR Ligand Domain	PFVNKQFNYKDPVNGVDIA
BoNT/B	2	M-PAR Ligand Domain	PVTINNFNYNDPIDNNNII
BoNT/C1	3	M-PAR Ligand Domain	PITINNFNYSDPVDNKNIL
BoNT/D	4	M-PAR Ligand Domain	TWPVKDFNYSDPVNDNDIL
BoNT/E	5	M-PAR Ligand Domain	PKINSFNYNDPVNDRTILY
BoNT/F	6	M-PAR Ligand Domain	PVAINSFNYNDPVNDDTIL
BoNT/G	7	M-PAR Ligand Domain	PVNIKXFNYNDPINDDII
TeNT	8	M-PAR Ligand Domain	PITINNFYSDPVNNDTII
The amino acid sequence displayed are as follows: BoNT/A, residues 2-20 of SEQ ID No: 1; BoNT/B, residues 2-20 of SEQ ID No: 2; BoNT/C1, residues v of SEQ ID No: 3; BoNT/D, residues 2-20 of SEQ ID No: 4; BoNT/E, residues 2-20 of SEQ ID No: 5; BoNT/F, residues 2-20 of SEQ ID No: 6; BoNT/G, residues 2-20 of SEQ ID No: 7; and TeNT, residues 2-20 of SEQ ID No: 8.			

[0108] In yet another embodiment, the location of the PAR ligand domain is located at the amino terminus of the modified Clostridial toxin. In such a location, the PAR ligand domain can bind to a ligand binding domain of a PAR; proteolytic cleavage is not necessary to unmask the PAR ligand domain. As used herein, the term "unmask" means that the amino terminus of a PAR ligand domain is free to bind to a ligand binding domain of a PAR. It is known in the art that when adding a polypeptide that is operationally-linked to the amino terminus of another polypeptide comprising the start methionine that this methionine residue can be deleted (Table 4). This is due to the fact that the added polypeptide will contain a new start methionine and that the original start methionine may reduce optimal expression of the fusion protein.

[0109] In yet another embodiment, the location of the PAR ligand domain is not located at the amino terminus of the modified Clostridial toxin. In such a location, the PAR ligand domain can not bind to a ligand binding domain of a PAR. The PAR ligand domain is considered masked because it is necessary to unmask a PAR ligand domain so that this domain can bind to a ligand binding domain of a PAR. As used herein, the term "masked" means that the amino terminus of a PAR ligand domain is unable to bind

Steward, L.E. *et al.*, Degradable Clostridial Toxins

to the ligand binding domain of a PAR. To unmask a PAR ligand domain of a modified Clostridial toxin, a protease cleavage site can be placed in front of the PAR ligand domain in such a manner that, upon cleavage with an appropriate protease, the masked PAR ligand domain becomes unmasked and is now capable of binding a PAR ligand binding domain. It is envisioned that any and all proteases that can cleave a modified Clostridial toxin disclosed in the present specification so as to unmask a PAR ligand domain can be used, including without limitation, a Clostridial toxin protease cleavage site found in the di-chain loop, a PAR protease cleavage site used to unmask the tethered ligand *in vivo*, and an exogenous protease cleavage site.

**[0110]** As mentioned above, a Clostridial toxin is converted from a single polypeptide form into a di-chain molecule by proteolytic cleavage. While the identity of the protease is currently unknown, the di-chain loop protease cleavage site for many Clostridial toxins has been determined. In BoNTs, cleavage at K448-A449 converts the single polypeptide form of BoNT/A into the di-chain form; cleavage at K441-A442 converts the single polypeptide form of BoNT/B into the di-chain form; cleavage at K449-T450 converts the single polypeptide form of BoNT/C1 into the di-chain form; cleavage at R445-D446 converts the single polypeptide form of BoNT/D into the di-chain form; cleavage at R422-K423 converts the single polypeptide form of BoNT/E into the di-chain form; cleavage at K439-A440 converts the single polypeptide form of BoNT/F into the di-chain form; and cleavage at K446-S447 converts the single polypeptide form of BoNT/G into the di-chain form. Proteolytic cleavage of the single polypeptide form of TeNT at A457-S458 results in the di-chain form. Such a di-chain loop protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. However, it should also be noted that additional cleavage sites within the di-chain loop also appear to be cleaved resulting in the generation of a small peptide fragment being lost. As a non-limiting example, BoNT/A single-chain polypeptide cleavage ultimately results in the loss of a ten amino acid fragment within the di-chain loop.

**[0111]** Thus, in an embodiment, proteolytic cleavage of an endogenous Clostridial toxin di-chain loop protease cleavage site is used to unmask a PAR ligand domain. In aspects of this embodiment, a PAR ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site or a TeNT di-chain loop protease cleavage site.

**[0112]** A wide variety of endogenous PAR proteases are known to cleave a PAR in such a manner as to unmask the tethered ligand and, therefore, can also be used to unmask the PAR ligand domain. The coagulant protease Thrombin is the physiological activator of PAR1, PAR3 and PAR4. Other PAR proteases, however, can also activate PAR receptors by proteolytic cleavage including, without limitation, APC, Cathepsin G, Factor VIIa, Factor Xa, Granzyme A, Gingipains-R, Plasmin and Trypsins (Table 2). PAR2 can also be activated by multiple proteases including, without limitation, Acrosien, Der P1, Der P3,

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Der P9, Factor VIIa, Factor Xa, Gingipains-R, MT-SP1, Proteinase-3, Trypsins and Trypsases (Table 2). It is envisioned that both endogenous protease cleavage sites found associated with a particular PAR ligand domain, as well as exogenous protease cleavage sites from other PAR ligand domains can be used to cleave a modified Clostridial toxin disclosed in the present specification in order to unmask the PAR ligand binding domain. Such a PAR protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. As a non-limiting example, a PAR1 ligand domain can be unmasked using the protease cleavage site associated with the *in vivo* PAR1 molecule, or a PAR1 ligand domain can be unmasked using the protease cleavage site associated with PAR2, PAR3 or PAR4 (Table 2). As another non-limiting example, a PAR2 ligand domain can be unmasked using the protease cleavage site associated with the *in vivo* PAR2 molecule, or a PAR2 ligand domain can be unmasked using the protease cleavage site associated with PAR1, PAR3 or PAR4 (Table 2). As still another non-limiting example, a PAR3 ligand domain can be unmasked using the protease cleavage site associated with the *in vivo* PAR3 molecule, or a PAR3 ligand domain can be unmasked using the protease cleavage site associated with PAR1, PAR2 or PAR4 (Table 2). As yet another non-limiting example, a PAR4 ligand domain can be unmasked using the protease cleavage site associated with the *in vivo* PAR4 molecule, or a PAR4 ligand domain can be unmasked using the protease cleavage site associated with PAR1, PAR2 or PAR3 (Table 2).

[0113] Thus, in an embodiment, proteolytic cleavage of an endogenous PAR1 protease cleavage site is used to unmask a PAR ligand domain. In aspects of this embodiment, a PAR ligand domain is unmasked by proteolytic cleavage of, *e.g.*, an APC protease cleavage site, a Factor Xa protease cleavage site, a Granzyme A protease cleavage site, a Gingipains-R protease cleavage site, a Thrombin protease cleavage site or a Trypsin protease cleavage site. In other aspects of this embodiment, a PAR1 protease cleavage site is cleaved by, *e.g.*, an APC protease, a Factor Xa protease, a Granzyme A protease, a Gingipains-R protease, a Thrombin protease or a Trypsin protease.

[0114] In another embodiment, proteolytic cleavage of an endogenous PAR2 protease cleavage site is used to unmask a PAR ligand domain. In aspects of this embodiment, a PAR ligand domain is unmasked by proteolytic cleavage of, *e.g.*, an Acrosien protease cleavage site, a Der P1 protease cleavage site, a Der P3 protease cleavage site, a Der P9 protease cleavage site, a Factor VIIa protease cleavage site, a Factor Xa protease cleavage site, a Gingipains-R protease cleavage site, a MT-SP1 protease cleavage site, a Proteinase-3 protease cleavage site, a Trypsin protease cleavage site or a Trypsase protease cleavage site. In other aspects of this embodiment, a PAR2 protease cleavage site is cleaved by, *e.g.*, an Acrosien protease, a Der P1 protease, a Der P3 protease, a Der P9 protease, a Factor VIIa protease, a Factor Xa protease, a Gingipains-R protease, a MT-SP1 protease, a Proteinase-3 protease, a Trypsin protease or a Trypsase protease.

[0115] In another embodiment, proteolytic cleavage of an endogenous PAR3 protease cleavage site is used to unmask a PAR ligand domain. In an aspect of this embodiment, a PAR ligand domain is

Steward, L.E. *et al.*, Degradable Clostridial Toxins

unmasked by proteolytic cleavage of, *e.g.*, a Thrombin protease cleavage site. In another aspect of this embodiment, a PAR3 protease cleavage site is cleaved by, *e.g.*, a Thrombin protease.

[0116] In another embodiment, proteolytic cleavage of an endogenous PAR4 protease cleavage site is used to unmask a PAR ligand domain. In aspects of this embodiment, a PAR ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a Cathepsin G protease cleavage site, a Factor VIIa protease cleavage site, a Factor Xa protease cleavage site, a Gingipains-R protease cleavage site, a Plasmin protease cleavage site, a Thrombin protease cleavage site or a Trypsin protease cleavage site. In other aspects of this embodiment, a PAR4 protease cleavage site is cleaved by, *e.g.*, a Cathepsin G protease, a Factor VIIa protease, a Factor Xa protease, a Gingipains-R protease, a Plasmin protease, a Thrombin protease or a Trypsin protease.

[0117] It is also envisioned that an exogenous protease cleavage site can be used to unmask a PAR ligand domain. Such an exogenous protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. Non-limiting examples of protease cleavage sites include, *e.g.*, an enterokinase cleavage site (Table 5); a Thrombin cleavage site (Table 5); a Factor Xa cleavage site (Table 5); a human rhinovirus 3C protease cleavage site (Table 4); a tobacco etch virus (TEV) protease cleavage site (Table 5); a dipeptidyl aminopeptidase cleavage site and a small ubiquitin-like modifier (SUMO)/ubiquitin-like protein-1 (ULP-1) protease cleavage site, such as, *e.g.*, MADSEVNQEAKPEVKP EVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEFAKRRQKGEMDSLRFYDGIHQADQTPEDLDMEDNDI IEAHREQIGG (SEQ ID. NO: 67). As a non-limiting example, a PAR1 ligand domain can be unmasked using a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site (Table 5). As another non-limiting example, a PAR2 ligand domain can be unmasked using a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site (Table 5). As still another non-limiting example, a PAR3 ligand domain can be unmasked using a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site (Table 5). As yet another non-limiting example, a PAR4 ligand domain can be unmasked using a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site (Table 5).

[0118] Thus, in an embodiment, proteolytic cleavage of an exogenous protease cleavage site is used to unmask a PAR ligand domain. In aspects of this embodiment, a PAR ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease

Steward, L.E. *et al.*, Degradable Clostridial Toxins

cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, a PAR protease cleavage site is cleaved by, *e.g.*, a bovine enterokinase protease, a Tobacco Etch Virus protease, a Human Rhinovirus 3C protease, a SUMO/ULP-1 protease, a Thrombin protease or a Factor Xa protease, thereby unmasking a PAR ligand domain.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Table 5. Exogenous Protease Cleavage Sites			
Protease Cleavage Site	Consensus Sequence	Non-limiting Examples	SEQ ID NO
Bovine enterokinase	DDDDK*	DDDDK*	50
Tobacco Etch Virus (TEV)	E P <sup>5</sup> P <sup>4</sup> YP <sup>2</sup> Q*(G/S), where P <sup>2</sup> , P <sup>4</sup> and P <sup>5</sup> can be any amino acid	ENLYFQ*G ENLYFQ*S ENIYTQ*G ENIYTQ*S ENIYLQ*G ENIYLQ*S ENVYFQ*G ENVYSQ*S ENVYSQ*G ENVYSQ*S	51 52 53 54 55 56 57 58 59 60
Human Rhinovirus 3C	P <sup>5</sup> P <sup>4</sup> LFQ*GP where P <sup>4</sup> is G, A, V, L, I, M, S or T and P <sup>5</sup> can any amino acid, with D or E preferred.	EALFQ*GP EVLFG*GP ELLFQ*GP DALFQ*GP DVLFQ*GP DLLFQ*GP	61 62 63 64 65 66
SUMO/ULP-1	Tertiary structure	polypeptide-G*	67
Thrombin	P <sup>3</sup> P <sup>2</sup> (R/K)*P <sup>1</sup> , where P <sup>3</sup> is any amino acid and P <sup>2</sup> or P <sup>1</sup> is G with the other position being any amino acid	GVR*G SAR*G SLR*G DGR*I QGK*I	68 69 70 71 72
Thrombin	P <sup>4</sup> P <sup>3</sup> P(R/K)*P <sup>1</sup> P <sup>2</sup> where P <sup>1</sup> and P <sup>2</sup> can be any amino acid except for acidic amino acids like D or E; and P <sup>3</sup> and P <sup>4</sup> are hydrophobic amino acids like F, L, I, Y, W, V, M, P, C or A	LVPR*GS LVPR*GS FIPR*TF VLPR*SF IVPR*SF IVPR*GY VVPR*GV VLPR*LI VMPR*SL MFPR*SL	73 74 75 76 77 78 79 80 81 82
Coagulation Factor Xa	I(E/D)GR*	IDGR* IEGR*	83 84
An asterisks (*) indicates the peptide bond that is cleaved by the indicated protease.			

[0119] In another embodiment, proteolytic cleavage of an exogenous protease cleavage site is used to unmask a PAR1 ligand domain. In aspects of this embodiment, a PAR1 ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, a PAR1 protease cleavage site is cleaved by, *e.g.*, a bovine enterokinase protease, a Tobacco Etch Virus protease, a Human Rhinovirus 3C protease, a SUMO/ULP-1 protease, a Thrombin protease or a Factor Xa protease, thereby unmasking a PAR1 ligand domain.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

[0120] In another embodiment, proteolytic cleavage of an exogenous protease cleavage site is used to unmask a PAR2 ligand domain. In aspects of this embodiment, a PAR2 ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, a PAR2 protease cleavage site is cleaved by, *e.g.*, a bovine enterokinase protease, a Tobacco Etch Virus protease, a Human Rhinovirus 3C protease, a SUMO/ULP-1 protease, a Thrombin protease or a Factor Xa protease, thereby unmasking a PAR2 ligand domain.

[0121] In still another embodiment, proteolytic cleavage of an exogenous protease cleavage site is used to unmask a PAR3 ligand domain. In aspects of this embodiment, a PAR3 ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, a PAR3 protease cleavage site is cleaved by, *e.g.*, a bovine enterokinase protease, a Tobacco Etch Virus protease, a Human Rhinovirus 3C protease, a SUMO/ULP-1 protease, a Thrombin protease or a Factor Xa protease, thereby unmasking a PAR3 ligand domain.

[0122] In another embodiment, proteolytic cleavage of an exogenous protease cleavage site is used to unmask a PAR4 ligand domain. In aspects of this embodiment, a PAR4 ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, a PAR4 protease cleavage site is cleaved by, *e.g.*, a bovine enterokinase protease, a Tobacco Etch Virus protease, a Human Rhinovirus 3C protease, a SUMO/ULP-1 protease, a Thrombin protease or a Factor Xa protease, thereby unmasking a PAR4 ligand domain.

[0123] It is understood that a modified Clostridial toxin disclosed in the present specification can optionally include one or more additional components. As a non-limiting example of an optional component, a modified Clostridial toxin can further comprise a flexible region comprising a flexible spacer. Non-limiting examples of a flexible spacer include, *e.g.*, a G-spacer GGGGS (SEQ ID NO: 48) or an A-spacer EAAAK (SEQ ID NO: 49). A flexible region comprising flexible spacers can be used to adjust the length of a polypeptide region in order to optimize a characteristic, attribute or property of a polypeptide. Such a flexible region is operably-linked in-frame to the modified Clostridial toxin as a fusion protein. As a non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be used to better expose a protease cleavage site thereby facilitating cleavage of that site by a protease. As another non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be used to better present a ligand domain, thereby facilitating the binding of that ligand domain to its binding domain on a receptor.



Steward, L.E. *et al.*, Degradable Clostridial Toxins

[0124] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise a flexible region comprising a flexible spacer. In another embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise flexible region comprising a plurality of flexible spacers in tandem. In aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at least 1 G-spacer, at least 2 G-spacers, at least 3 G-spacers, at least 4 G-spacers or at least 5 G-spacers. In other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at most 1 G-spacer, at most 2 G-spacers, at most 3 G-spacers, at most 4 G-spacers or at most 5 G-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at least 1 A-spacer, at least 2 A-spacers, at least 3 A-spacers, at least 4 A-spacers or at least 5 A-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at most 1 A-spacer, at most 2 A-spacers, at most 3 A-spacers, at most 4 A-spacers or at most 5 A-spacers. In another aspect of this embodiment, a modified Clostridial toxin can comprise a flexible region comprising one or more copies of the same flexible spacers, one or more copies of different flexible-spacer regions, or any combination thereof.

[0125] As another non-limiting example of an optional component, a modified Clostridial toxin can further comprise an epitope-binding region. An epitope-binding region can be used in a wide variety of procedures involving, *e.g.*, protein purification and protein visualization. Such an epitope-binding region is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. Non-limiting examples of an epitope-binding region include, *e.g.*, FLAG, Express™, human Influenza virus hemagglutinin (HA), human p62<sup>c-Myc</sup> protein (c-MYC), Vesicular Stomatitis Virus Glycoprotein (VSV-G), glycoprotein-D precursor of Herpes simplex virus (HSV), V5, and AU1; affinity-binding, such as, *e.g.*, polyhistidine (HIS), streptavidin binding peptide (strep), and biotin or a biotinylation sequence; peptide-binding regions, such as, *e.g.*, the glutathione binding domain of glutathione-S-transferase, the calmodulin binding domain of the calmodulin binding protein, and the maltose binding domain of the maltose binding protein. Non-limiting examples of specific protocols for selecting, making and using an appropriate binding peptide are described in, *e.g.*, Epitope Tagging, pp. 17.90-17.93 (Sambrook and Russell, eds., Molecular Cloning A Laboratory Manual, Vol. 3, 3<sup>rd</sup> ed. 2001); Antibodies: A Laboratory Manual (Edward Harlow & David Lane, eds., Cold Spring Harbor Laboratory Press, 2<sup>nd</sup> ed. 1998); and Using Antibodies: A Laboratory Manual: Portable Protocol No. 1 (Edward Harlow & David Lane, Cold Spring Harbor Laboratory Press, 1998). In addition, non-limiting examples of binding peptides as well as well-characterized reagents, conditions and protocols are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0126] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise an epitope-binding region. In another embodiment, a modified Clostridial toxin disclosed

Steward, L.E. *et al.*, Degradable Clostridial Toxins

in the present specification can further comprises a plurality of epitope-binding regions. In aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at least 1 epitope-binding region, at least 2 epitope-binding regions, at least 3 epitope-binding regions, at least 4 epitope-binding regions or at least 5 epitope-binding regions. In other aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at most 1 epitope-binding region, at most 2 epitope-binding regions, at most 3 epitope-binding regions, at most 4 epitope-binding regions or at most 5 epitope-binding regions. In another aspect of this embodiment, a modified Clostridial toxin can comprise one or more copies of the same epitope-binding region, one or more copies of different epitope-binding regions, or any combination thereof. The location of an epitope-binding region can be in various positions, including, without limitation, at the amino terminus of a modified Clostridial toxin, within a modified Clostridial toxin, or at the carboxyl terminus of a modified Clostridial toxin.

**[0127]** As still another non-limiting example of an optional component, a modified Clostridial toxin can further comprise an exogenous protease cleavage site. An exogenous protease cleavage site can be used in a wide variety of procedures involving, *e.g.*, removal of an epitope-binding region by proteolytic cleavage, conversion of a Clostridial toxin single chain polypeptide into the di-chain form or, as mentioned above, unmasking of a PAR ligand domain. Such an exogenous protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. Non-limiting examples of protease cleavage sites include, *e.g.*, an enterokinase cleavage site (Table 5); a Thrombin cleavage site (Table 5); a Factor Xa cleavage site (Table 5); a human rhinovirus 3C protease cleavage site (Table 4); a tobacco etch virus (TEV) protease cleavage site (Table 5); a dipeptidyl aminopeptidase cleavage site and a small ubiquitin-like modifier (SUMO)/ubiquitin-like protein-1(ULP-1) protease cleavage site, such as, *e.g.*, MADSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQ GKEMDSL RFLY DGIRIQADQTPEDLDMEDNDIIEAHREQIGG (SEQ ID. NO: 67).

**[0128]** Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise an exogenous protease cleavage site. In another embodiment, a modified Clostridial toxin disclosed in the present specification can further comprises a plurality of exogenous protease cleavage sites. In aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at least 1 exogenous protease cleavage site, at least 2 exogenous protease cleavage sites, at least 3 exogenous protease cleavage sites, at least 4 exogenous protease cleavage sites or at least 5 exogenous protease cleavage sites. In other aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at most 1 exogenous protease cleavage site, at most 2 exogenous protease cleavage sites, at most 3 exogenous protease cleavage sites, at most 4 exogenous protease cleavage sites or at most 5 exogenous protease cleavage sites. In another aspect of this embodiment, a modified Clostridial toxin can comprise one or more copies of the same exogenous protease cleavage site, one or more copies of different exogenous protease cleavage sites, or any combination thereof.

[0129] The location of an exogenous protease cleavage site may be in a variety of positions, including, without limitation, between an epitope-binding region and a modified Clostridial toxin in order to facilitate removal of the epitope-binding region by proteolytic cleavage or within the di-chain loop of the modified Clostridial toxin in order to facilitate the conversion of the single-chain polypeptide form of the toxin into the di-chain form.

[0130] It is envisioned that an exogenous protease cleavage site can be used to remove an epitope-binding region. As mentioned above, epitope binding regions can be used in protein purification procedures and it is often desirable to remove such epitope binding regions after the protein is purified. A common way of doing so is to have a protease cleavage site in between the protein of interest and the epitope binding region, whereby proteolytic cleavage of the protease cleavage site separates the protein of interest from the epitope binding region. Non-limiting examples of protease cleavage sites used for the removal of epitope-binding regions as well as well-characterized proteases, reagents, conditions and protocols are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. The selection, making and use of an appropriate protease cleavage site are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0131] Thus, in an embodiment, an exogenous protease cleavage site is located between an epitope-binding peptide and a modified Clostridial toxin. In other aspects of this embodiment, a bovine enterokinase cleavage site is located between an epitope-binding region and a modified Clostridial toxin, a Tobacco Etch Virus protease cleavage site is located between an epitope-binding region and a modified Clostridial toxin, a Human Rhinovirus 3C protease cleavage site is located between an epitope-binding region and a modified Clostridial toxin, a SUMO/ULP-1 protease cleavage site is located between an epitope-binding region and a modified Clostridial toxin, a Thrombin protease cleavage site is located between an epitope-binding region and a modified Clostridial toxin, or a Coagulation Factor Xa protease cleavage site is located between an epitope-binding region and a modified Clostridial toxin. In other aspects of the embodiment, the bovine enterokinase protease cleavage site located between an epitope-binding region and a modified Clostridial toxin comprises SEQ ID NO: 50. In other aspects of the embodiment, the Tobacco Etch Virus protease cleavage site located between an epitope-binding region and a modified Clostridial toxin comprises SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60. In still other aspects of the embodiment, the Human Rhinovirus 3C protease cleavage site located between an epitope-binding region and a modified Clostridial toxin comprises SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of the embodiment, the SUMO/ULP-1 protease cleavage site located between an epitope-binding region and a modified Clostridial toxin comprises SEQ ID NO: 67. In further other aspects of the embodiment, the Thrombin protease cleavage site located between an epitope-binding region and a modified Clostridial

Steward, L.E. *et al.*, Degradable Clostridial Toxins

toxin comprises SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81 or SEQ ID NO: 82. In other aspects of the embodiment, the Coagulation Factor Xa protease cleavage site located between an epitope-binding region and a modified Clostridial toxin comprises SEQ ID NO: 83 or SEQ ID NO: 84.

Table 6: Di-chain Loop Region				
Toxin	SEQ ID NO	Light Chain Region	Di-chain Loop Protease Cleavage Site Region	Heavy Chain Region
BoNT/A	1	NMNFTKLKNFTGLFEFYKLL	CVRGIITSKTKSLDKGYNK*-----ALNDLC	IKVNNWDL
BoNT/B	2	KQAYEEISKEHLAVYKIQM	CKSVK*-----APGIC	IDVDNEDL
BoNT/C1	3	PALRKVNPNMMLYLFTKF	CHKAIDGRSLYNK*-----TLDC	RELLVKNTDL
BoNT/D	4	PALQKLSSESVDLFTKV	CLRLTKNSR*-----DDSTC	IKVKNNRL
BoNT/E	5	IITPITGRGLVKKIIRF	CKNIVSVKGIR*-----KSIC	IEINNDEL
BoNT/F	6	IIDSIPDKGLVEKIVKF	CKSVIPRKGTK*-----APRLC	IRVNNSEL
BoNT/G	7	KEAYEEISLEHLVIYRIAM	CKPVMYKNTGK*-----SEQC	IIVNNEDL
TeNT	8	TNAFRNVDGSGLVSKLIGL	CKKIIPPTNIRENLYNRTA*SLTDLGGELC	IKIKNEDL

The amino acid sequence displayed are as follows: BoNT/A, residues 325-462 of SEQ ID No: 1; BoNT/B, residues 332-454 of SEQ ID No: 2; BoNT/C1, residues 334-463 of SEQ ID No: 3; BoNT/D, residues 334-458 of SEQ ID No: 4; BoNT/E, residues 311-434 of SEQ ID No: 5; BoNT/F, residues 328-453 of SEQ ID No: 6; BoNT/G, residues 331-458 of SEQ ID No: 7; and TeNT, residues 334-474 of SEQ ID No: 8. An asterisks (\*) indicates the peptide bond that is cleaved by a Clostridial toxin protease.

[0132] It is envisioned that an exogenous protease cleavage site can be used to convert the single-chain polypeptide form of a modified Clostridial toxin disclosed in the present specification into the di-chain form. As mentioned above, Clostridial toxins are translated as a single-chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulphide bond and noncovalent interactions. While the naturally-occurring protease is currently not known, cleavage occurs within the di-chain loop region between the two cysteine residues that form the disulfide bridge (Table 6). Replacement of the naturally-occurring protease cleavage site with an exogenous protease cleavage site will enable cleavage of a modified Clostridial toxin disclosed in the present specification when expressed in an organism that does not produce the endogenous Clostridial protease used to cleave the di-chain loop region of a toxin.

[0133] Thus in an embodiment, an exogenous protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In aspects of this embodiment, a bovine enterokinase cleavage site is located within the di-chain loop of a modified Clostridial toxin, a Tobacco Etch Virus protease cleavage site is located within the di-chain loop of a modified Clostridial toxin, a Human Rhinovirus 3C protease cleavage site is located within the di-chain loop of a modified Clostridial toxin, a SUMO/ULP-1 protease

Steward, L.E. *et al.*, Degradable Clostridial Toxins

cleavage site is located within the di-chain loop of a modified Clostridial toxin, a Thrombin protease cleavage site is located within the di-chain loop of a modified Clostridial toxin, or a Coagulation Factor Xa protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, the bovine enterokinase protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 50. In other aspects of the embodiment, the Tobacco Etch Virus protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60. In still other aspects of the embodiment, the Human Rhinovirus 3C protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of the embodiment, the SUMO/ULP-1 protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 67. In further other aspects of the embodiment, the Thrombin protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81 or SEQ ID NO: 82. In other aspects of the embodiment, the Coagulation Factor Xa protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 83 or SEQ ID NO: 84.

**[0134]** Aspects of the present invention provide, in part modified Clostridial toxins. Non-limiting examples of Clostridial toxin modifications disclosed in the present specification include, *e.g.*, addition of a PAR ligand domain, addition of a protease cleavage site, rearrangement of the enzymatic, translocation and binding domains, addition of a spacer region and addition of an epitope-binding region. It is understood that all such modifications do not substantially affect the ability of a Clostridial toxin to intoxicate a cell. As used herein, the term "do not substantially affect" means a Clostridial toxin can still execute the overall cellular mechanism whereby a Clostridial toxin enters a neuron and inhibits neurotransmitter release and encompasses the binding of a Clostridial toxin to a low or high affinity receptor complex, the internalization of the toxin/receptor complex, the translocation of the Clostridial toxin light chain into the cytoplasm and the enzymatic modification of a Clostridial toxin substrate. In aspects of this embodiment, the modified Clostridial toxin is, *e.g.*, at least 10% as toxic as a naturally-occurring Clostridial toxin, at least 20% as toxic as a naturally-occurring Clostridial toxin, at least 30% as toxic as a naturally-occurring Clostridial toxin, at least 40% as toxic as a naturally-occurring Clostridial toxin, at least 50% as toxic as a naturally-occurring Clostridial toxin, at least 60% as toxic as a naturally-occurring Clostridial toxin, at least 70% as toxic as a naturally-occurring Clostridial toxin, at least 80% as toxic as a naturally-occurring Clostridial toxin, at least 90% as toxic as a naturally-occurring Clostridial toxin or at least 95% as toxic as a naturally-occurring Clostridial toxin. In aspects of this embodiment, the modified Clostridial toxin is, *e.g.*, at most 10% as toxic as a naturally-occurring Clostridial toxin, at most 20% as toxic as a naturally-occurring Clostridial toxin, at most 30% as toxic as a naturally-occurring Clostridial toxin, at most 40% as toxic as a naturally-occurring Clostridial toxin, at most 50% as toxic as a

Steward, L.E. *et al.*, Degradable Clostridial Toxins

naturally-occurring Clostridial toxin, at most 60% as toxic as a naturally-occurring Clostridial toxin, at most 70% as toxic as a naturally-occurring Clostridial toxin, at most 80% as toxic as a naturally-occurring Clostridial toxin, at most 90% as toxic as a naturally-occurring Clostridial toxin or at most 95% as toxic as a naturally-occurring Clostridial toxin.

**[0135]** Aspects of the present invention provide, in part polynucleotide molecules. As used herein, the term "polynucleotide molecule" is synonymous with "nucleic acid molecule" and means a polymeric form of nucleotides, such as, *e.g.*, ribonucleotides and deoxyribonucleotides, of any length. It is envisioned that any and all polynucleotide molecules that can encode a modified Clostridial toxin disclosed in the present specification can be useful, including, without limitation naturally-occurring and non-naturally-occurring DNA molecules and naturally-occurring and non-naturally-occurring RNA molecules. Non-limiting examples of naturally-occurring and non-naturally-occurring DNA molecules include single-stranded DNA molecules, double-stranded DNA molecules, genomic DNA molecules, cDNA molecules, vector constructs, such as, *e.g.*, plasmid constructs, phagmid constructs, bacteriophage constructs, retroviral constructs and artificial chromosome constructs. Non-limiting examples of naturally-occurring and non-naturally-occurring RNA molecules include single-stranded RNA, double stranded RNA and mRNA.

**[0136]** Thus, in an embodiment, a polynucleotide molecule encodes a Clostridial toxin comprises a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin binding domain. In an aspect of this embodiment, a polynucleotide molecule encodes a Clostridial toxin comprises a naturally occurring Clostridial toxin variant, such as, *e.g.*, a Clostridial toxin isoform or a Clostridial toxin subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a Clostridial toxin comprises a non-naturally occurring Clostridial toxin variant, such as, *e.g.*, a conservative Clostridial toxin variant, a non-conservative Clostridial toxin variant or an active Clostridial toxin fragment, or any combination thereof. In another aspect of this embodiment, a polynucleotide molecule encodes a Clostridial toxin comprises a Clostridial toxin enzymatic domain or an active fragment thereof, a Clostridial toxin translocation domain or an active fragment thereof, a Clostridial toxin binding domain or an active fragment thereof, or any combination thereof. In other aspects of this embodiment, a Clostridial toxins comprises a BoNT/A, a BoNT/B, a BoNT/C1, a BoNT/D, a BoNT/E, a BoNT/F, a BoNT/G or a TeNT.

**[0137]** In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/A. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a BoNT/A enzymatic domain, a BoNT/A translocation domain and a BoNT/A binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising SEQ ID NO: 1. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a naturally occurring BoNT/A variant, such as, *e.g.*, a BoNT/A isoform or a BoNT/A subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a naturally occurring BoNT/A variant of SEQ ID NO: 1, such as, *e.g.*, a BoNT/A isoform of SEQ ID NO: 1 or a BoNT/A subtype of SEQ

ID NO: 1. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a non-naturally occurring BoNT/A variant, such as, *e.g.*, a conservative BoNT/A variant, a non-conservative BoNT/A variant or an active BoNT/A fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a non-naturally occurring BoNT/A variant of SEQ ID NO: 1, such as, *e.g.*, a conservative BoNT/A variant of SEQ ID NO: 1, a non-conservative BoNT/A variant of SEQ ID NO: 1 or an active BoNT/A fragment of SEQ ID NO: 1, or any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a BoNT/A enzymatic domain or an active fragment thereof, a BoNT/A translocation domain or an active fragment thereof, a BoNT/A binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/A comprising a BoNT/A enzymatic domain of amino acids 1-448 from SEQ ID NO: 1 or an active fragment thereof, a BoNT/A translocation domain of amino acids 449-860 from SEQ ID NO: 1 or an active fragment thereof, a BoNT/A binding domain of amino acids 861-1296 from SEQ ID NO: 1 or an active fragment thereof, and any combination thereof.

**[0138]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 1, at least 75% amino acid identity with the SEQ ID NO: 1, at least 80% amino acid identity with SEQ ID NO: 1, at least 85% amino acid identity with SEQ ID NO: 1, at least 90% amino acid identity with SEQ ID NO: 1 or at least 95% amino acid identity with SEQ ID NO: 1. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 1, at most 75% amino acid identity with the SEQ ID NO: 1, at most 80% amino acid identity with SEQ ID NO: 1, at most 85% amino acid identity with SEQ ID NO: 1, at most 90% amino acid identity with SEQ ID NO: 1 or at most 95% amino acid identity with SEQ ID NO: 1.

**[0139]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 1. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 1. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 1. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 1. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 1. In other

Steward, L.E. *et al.*, Degradable Clostridial Toxins

aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 1.

[0140] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 1. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 1. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 1. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 1. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 1. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 1.

[0141] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/B. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a BoNT/B enzymatic domain, a BoNT/B translocation domain and a BoNT/B binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising SEQ ID NO: 2. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a naturally occurring BoNT/B variant, such as, *e.g.*, a BoNT/B isoform or a BoNT/B subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a naturally occurring BoNT/B variant of SEQ ID NO: 2, such as, *e.g.*, a BoNT/B isoform of SEQ ID NO: 2 or a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a non-naturally occurring BoNT/B variant, such as, *e.g.*, a conservative BoNT/B variant, a non-conservative BoNT/B variant or an active BoNT/B fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a non-naturally occurring BoNT/B variant of SEQ ID NO: 2, such as, *e.g.*, a conservative BoNT/B variant of SEQ ID NO: 2, a non-conservative BoNT/B variant of SEQ ID NO: 2 or an active BoNT/B fragment of SEQ ID NO: 2, or any combination thereof. In yet another aspect of this embodiment, a BoNT/B comprising a BoNT/B enzymatic domain or an active fragment thereof, a BoNT/B translocation domain or active fragment thereof, a BoNT/B binding domain or active fragment thereof, and any combination thereof. In



Steward, L.E. *et al.*, Degradable Clostridial Toxins

yet another aspect of this embodiment, a BoNT/B comprising a BoNT/B enzymatic domain of amino acids 1-441 from SEQ ID NO: 2 or active fragment thereof, a BoNT/B translocation domain of amino acids 442-847 from SEQ ID NO: 2 or active fragment thereof, a BoNT/B binding domain of amino acids 848-1291 from SEQ ID NO: 2 or active fragment thereof, and any combination thereof.

**[0142]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 2, at least 75% amino acid identity with the SEQ ID NO: 2, at least 80% amino acid identity with SEQ ID NO: 2, at least 85% amino acid identity with SEQ ID NO: 2, at least 90% amino acid identity with SEQ ID NO: 2 or at least 95% amino acid identity with SEQ ID NO: 2. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 2, at most 75% amino acid identity with the SEQ ID NO: 2, at most 80% amino acid identity with SEQ ID NO: 2, at most 85% amino acid identity with SEQ ID NO: 2, at most 90% amino acid identity with SEQ ID NO: 2 or at most 95% amino acid identity with SEQ ID NO: 2.

**[0143]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 2. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 2. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 2.

**[0144]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 2. In yet other aspects of this embodiment, a

Steward, L.E. *et al.*, Degradable Clostridial Toxins

polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 2. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 2.

[0145] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/C1. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain and a BoNT/C1 binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising SEQ ID NO: 3. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a naturally occurring BoNT/C1 variant, such as, *e.g.*, a BoNT/C1 isoform or a BoNT/C1 subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a naturally occurring BoNT/C1 variant of SEQ ID NO: 3, such as, *e.g.*, a BoNT/C1 isoform of SEQ ID NO: 3 or a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a non-naturally occurring BoNT/C1 variant, such as, *e.g.*, a conservative BoNT/C1 variant, a non-conservative BoNT/C1 variant or an active BoNT/C1 fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a non-naturally occurring BoNT/C1 variant of SEQ ID NO: 3, such as, *e.g.*, a conservative BoNT/C1 variant of SEQ ID NO: 3, a non-conservative BoNT/C1 variant of SEQ ID NO: 3 or an active BoNT/C1 fragment of SEQ ID NO: 3, or any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a BoNT/C1 enzymatic domain or active fragment thereof, a BoNT/C1 translocation domain or active fragment thereof, a BoNT/C1 binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a BoNT/C1 enzymatic domain of amino acid 1-449 from SEQ ID NO: 3 or active fragment thereof, a BoNT/C1 translocation domain of amino acids 450-855 from SEQ ID NO: 3 or active fragment thereof, a BoNT/C1 binding domain of amino acids 856-1291 from SEQ ID NO: 3 or active fragment thereof, and any combination thereof.

[0146] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 3, at least 75% amino acid identity with the SEQ ID NO: 3, at least 80% amino acid identity with SEQ ID NO: 3, at least 85% amino acid identity with SEQ ID NO: 3, at least 90% amino acid identity with SEQ ID NO: 3 or at least 95%

Steward, L.E. *et al.*, Degradable Clostridial Toxins

amino acid identity with SEQ ID NO: 3. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 3, at most 75% amino acid identity with the SEQ ID NO: 3, at most 80% amino acid identity with SEQ ID NO: 3, at most 85% amino acid identity with SEQ ID NO: 3, at most 90% amino acid identity with SEQ ID NO: 3 or at most 95% amino acid identity with SEQ ID NO: 3.

**[0147]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 3. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 3. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 3.

**[0148]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 3. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 3. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at

Steward, L.E. *et al.*, Degradable Clostridial Toxins

least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 3.

[0149] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/D. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a BoNT/D enzymatic domain, a BoNT/D translocation domain and a BoNT/D binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising SEQ ID NO: 4. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a naturally occurring BoNT/D variant, such as, *e.g.*, a BoNT/D isoform or a BoNT/D subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a naturally occurring BoNT/D variant of SEQ ID NO: 4, such as, *e.g.*, a BoNT/D isoform of SEQ ID NO: 4 or a BoNT/D subtype of SEQ ID NO: 4. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a non-naturally occurring BoNT/D variant, such as, *e.g.*, a conservative BoNT/D variant, a non-conservative BoNT/D variant or an active BoNT/D fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a non-naturally occurring BoNT/D variant of SEQ ID NO: 4, such as, *e.g.*, a conservative BoNT/D variant of SEQ ID NO: 4, a non-conservative BoNT/D variant of SEQ ID NO: 4 or an active BoNT/D fragment of SEQ ID NO: 4, or any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a BoNT/D enzymatic domain or an active fragment thereof, a BoNT/D translocation domain or an active fragment thereof, a BoNT/D binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/D comprising a BoNT/D enzymatic domain of amino acids 1-442 from SEQ ID NO: 4 or an active fragment thereof, a BoNT/D translocation domain of amino acids 443-851 from SEQ ID NO: 4 or an active fragment thereof, a BoNT/D binding domain of amino acids 852-1276 from SEQ ID NO: 4 or an active fragment thereof, and any combination thereof.

[0150] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 4, at least 75% amino acid identity with the SEQ ID NO: 4, at least 80% amino acid identity with SEQ ID NO: 4, at least 85% amino acid identity with SEQ ID NO: 4, at least 90% amino acid identity with SEQ ID NO: 4 or at least 95% amino acid identity with SEQ ID NO: 4. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 4, at most 75% amino acid identity with the SEQ ID NO: 4, at most 80% amino acid identity with SEQ ID NO: 4, at most 85% amino acid identity with SEQ ID NO: 4, at most 90% amino acid identity with SEQ ID NO: 4 or at most 95% amino acid identity with SEQ ID NO: 4.

[0151] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 4. In other aspects of

this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 4. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 4. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 4. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 4. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 4.

[0152] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 4. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 4. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 4. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 4. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 4. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 4.

[0153] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/E. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a BoNT/E enzymatic domain, a BoNT/E translocation domain and a BoNT/E binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising SEQ ID NO: 5. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a naturally occurring BoNT/E variant, such as, *e.g.*, a BoNT/E isoform or a BoNT/E subtype. In another aspect of

Steward, L.E. *et al.*, Degradable Clostridial Toxins

this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a naturally occurring BoNT/E variant of SEQ ID NO: 5, such as, *e.g.*, a BoNT/E isoform of SEQ ID NO: 5 or a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a non-naturally occurring BoNT/E variant, such as, *e.g.*, a conservative BoNT/E variant, a non-conservative BoNT/E variant or an active BoNT/E fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a non-naturally occurring BoNT/E variant of SEQ ID NO: 5, such as, *e.g.*, a conservative BoNT/E variant of SEQ ID NO: 5, a non-conservative BoNT/E variant of SEQ ID NO: 5 or an active BoNT/E fragment of SEQ ID NO: 5, or any combination thereof. In yet another aspect of this embodiment, a BoNT/E comprising a BoNT/E enzymatic domain or an active fragment thereof, a BoNT/E translocation domain or active fragment thereof, a BoNT/E binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a BoNT/E comprising a BoNT/E enzymatic domain of amino acids 1-422 from SEQ ID NO: 5 or active fragment thereof, a BoNT/E translocation domain of amino acids 423-834 from SEQ ID NO: 5 or active fragment thereof, a BoNT/E binding domain of amino acids 835-1252 from SEQ ID NO: 5 or active fragment thereof, and any combination thereof.

**[0154]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 5, at least 75% amino acid identity with the SEQ ID NO: 5, at least 80% amino acid identity with SEQ ID NO: 5, at least 85% amino acid identity with SEQ ID NO: 5, at least 90% amino acid identity with SEQ ID NO: 5 or at least 95% amino acid identity with SEQ ID NO: 5. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 5, at most 75% amino acid identity with the SEQ ID NO: 5, at most 80% amino acid identity with SEQ ID NO: 5, at most 85% amino acid identity with SEQ ID NO: 5, at most 90% amino acid identity with SEQ ID NO: 5 or at most 95% amino acid identity with SEQ ID NO: 5.

**[0155]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 5. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 5. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20,

Steward, L.E. *et al.*, Degradable Clostridial Toxins

30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 5.

**[0156]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 5. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 5. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 5.

**[0157]** In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/F. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a BoNT/F enzymatic domain, a BoNT/F translocation domain and a BoNT/F binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising SEQ ID NO: 6. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a naturally occurring BoNT/F variant, such as, *e.g.*, a BoNT/F isoform or a BoNT/F subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a naturally occurring BoNT/F variant of SEQ ID NO: 6, such as, *e.g.*, a BoNT/F isoform of SEQ ID NO: 6 or a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a non-naturally occurring BoNT/F variant, such as, *e.g.*, a conservative BoNT/F variant, a non-conservative BoNT/F variant or an active BoNT/F fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a non-naturally occurring BoNT/F variant of SEQ ID NO: 6, such as, *e.g.*, a conservative BoNT/F variant of SEQ ID NO: 6, a non-conservative BoNT/F variant of SEQ ID NO: 6 or an active BoNT/F fragment of SEQ ID NO: 6, or any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a BoNT/F enzymatic domain or active fragment thereof, a BoNT/F translocation

Steward, L.E. et al., Degradable Clostridial Toxins

domain or active fragment thereof, a BoNT/F binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a BoNT/F enzymatic domain of amino acid 1-436 from SEQ ID NO: 6 or active fragment thereof, a BoNT/F translocation domain of amino acids 437-852 from SEQ ID NO: 6 or active fragment thereof, a BoNT/F binding domain of amino acids 853-1274 from SEQ ID NO: 6 or active fragment thereof, and any combination thereof.

[0158] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 6, at least 75% amino acid identity with the SEQ ID NO: 6, at least 80% amino acid identity with SEQ ID NO: 6, at least 85% amino acid identity with SEQ ID NO: 6, at least 90% amino acid identity with SEQ ID NO: 6 or at least 95% amino acid identity with SEQ ID NO: 6. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 6, at most 75% amino acid identity with the SEQ ID NO: 6, at most 80% amino acid identity with SEQ ID NO: 6, at most 85% amino acid identity with SEQ ID NO: 6, at most 90% amino acid identity with SEQ ID NO: 6 or at most 95% amino acid identity with SEQ ID NO: 6.

[0159] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 6. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 6. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 6.

[0160] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least



Steward, L.E. *et al.*, Degradable Clostridial Toxins

one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 6. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 6. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 6.

**[0161]** In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/G. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a BoNT/G enzymatic domain, a BoNT/G translocation domain and a BoNT/G binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising SEQ ID NO: 7. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a naturally occurring BoNT/G variant, such as, *e.g.*, a BoNT/G isoform or a BoNT/G subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a naturally occurring BoNT/G variant of SEQ ID NO: 7, such as, *e.g.*, a BoNT/G isoform of SEQ ID NO: 7 or a BoNT/G subtype of SEQ ID NO: 7. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a non-naturally occurring BoNT/G variant, such as, *e.g.*, a conservative BoNT/G variant, a non-conservative BoNT/G variant or an active BoNT/G fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a non-naturally occurring BoNT/G variant of SEQ ID NO: 7, such as, *e.g.*, a conservative BoNT/G variant of SEQ ID NO: 7, a non-conservative BoNT/G variant of SEQ ID NO: 7 or an active BoNT/G fragment of SEQ ID NO: 7, or any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a BoNT/G enzymatic domain or an active fragment thereof, a BoNT/G translocation domain or an active fragment thereof, a BoNT/G binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/G comprising a BoNT/G enzymatic domain of amino acids 1-442 from SEQ ID NO: 7 or an active fragment thereof, a BoNT/G translocation domain of amino acids 443-852 from SEQ ID NO: 7 or an active fragment thereof, a BoNT/G binding domain of amino acids 853-1297 from SEQ ID NO: 7 or an active fragment thereof, and any combination thereof.

**[0162]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 7, at least 75% amino acid

Steward, L.E. *et al.*, Degradable Clostridial Toxins

identity with the SEQ ID NO: 7, at least 80% amino acid identity with SEQ ID NO: 7, at least 85% amino acid identity with SEQ ID NO: 7, at least 90% amino acid identity with SEQ ID NO: 7 or at least 95% amino acid identity with SEQ ID NO: 7. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 7, at most 75% amino acid identity with the SEQ ID NO: 7, at most 80% amino acid identity with SEQ ID NO: 7, at most 85% amino acid identity with SEQ ID NO: 7, at most 90% amino acid identity with SEQ ID NO: 7 or at most 95% amino acid identity with SEQ ID NO: 7.

**[0163]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 7. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 7. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 7.

**[0164]** In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 7. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 7. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50,

100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 7.

**[0165]** In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a TeNT. In an aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising a TeNT enzymatic domain, a TeNT translocation domain and a TeNT binding domain. In an aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising SEQ ID NO: 8. In another aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising a naturally occurring TeNT variant, such as, *e.g.*, a TeNT isoform or a TeNT subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising a naturally occurring TeNT variant of SEQ ID NO: 8, such as, *e.g.*, a TeNT isoform of SEQ ID NO: 8 or a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising a non-naturally occurring TeNT variant, such as, *e.g.*, a conservative TeNT variant, a non-conservative TeNT variant or an active TeNT fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising a non-naturally occurring TeNT variant of SEQ ID NO: 8, such as, *e.g.*, a conservative TeNT variant of SEQ ID NO: 8, a non-conservative TeNT variant of SEQ ID NO: 8 or an active TeNT fragment of SEQ ID NO: 8, or any combination thereof. In yet another aspect of this embodiment, a TeNT comprising a TeNT enzymatic domain or an active fragment thereof, a TeNT translocation domain or active fragment thereof, a TeNT binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a TeNT comprising a TeNT enzymatic domain of amino acids 1-441 from SEQ ID NO: 8 or active fragment thereof, a TeNT translocation domain of amino acids 442-870 from SEQ ID NO: 8 or active fragment thereof, a TeNT binding domain of amino acids 871-1315 from SEQ ID NO: 8 or active fragment thereof, and any combination thereof.

**[0166]** In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 8, at least 75% amino acid identity with the SEQ ID NO: 8, at least 80% amino acid identity with SEQ ID NO: 8, at least 85% amino acid identity with SEQ ID NO: 8, at least 90% amino acid identity with SEQ ID NO: 8 or at least 95% amino acid identity with SEQ ID NO: 8. In yet other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 8, at most 75% amino acid identity with the SEQ ID NO: 8, at most 80% amino acid identity with SEQ ID NO: 8, at most 85% amino acid identity with SEQ ID NO: 8, at most 90% amino acid identity with SEQ ID NO: 8 or at most 95% amino acid identity with SEQ ID NO: 8.

**[0167]** In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50,

100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 8. In yet other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 8. In still other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 8.

[0168] In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 8. In yet other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 8. In still other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 8.

[0169] In still another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a naturally occurring PAR ligand domain variant, such as, *e.g.*, a PAR ligand domain isoform or a PAR ligand domain subtype. In another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a non-naturally occurring PAR ligand domain variant, such as, *e.g.*, a conservative PAR ligand domain variant, a non-conservative PAR ligand domain variant or a PAR ligand domain peptidomimetic, or any combination thereof.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

[0170] In still another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a PAR1 ligand domain. In an aspect of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising SEQ ID NO: 13. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a naturally occurring PAR1 ligand domain variant, such as, *e.g.*, a PAR1 ligand domain isoform or a PAR1 ligand domain subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a naturally occurring PAR1 ligand domain variant of SEQ ID NO: 13, such as, *e.g.*, a PAR1 ligand domain isoform of SEQ ID NO: 13 or a PAR1 ligand domain subtype of SEQ ID NO: 13. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a non-naturally occurring PAR1 ligand domain variant, such as, *e.g.*, a conservative PAR1 ligand domain variant, a non-conservative PAR1 ligand domain variant or a PAR1 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a non-naturally occurring PAR1 ligand domain variant of SEQ ID NO: 13, such as, *e.g.*, a conservative PAR1 ligand domain variant of SEQ ID NO: 13, a non-conservative PAR1 ligand domain variant of SEQ ID NO: 13 or a PAR1 ligand domain peptidomimetic of SEQ ID NO: 13, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22 or SEQ ID NO: 23.

[0171] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 13, at least 67% amino acid identity with the SEQ ID NO: 13, or at least 83% amino acid identity with SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 13, at most 67% amino acid identity with the SEQ ID NO: 13, at most 83% amino acid identity with SEQ ID NO: 13.

[0172] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 13. In

Steward, L.E. *et al.*, Degradable Clostridial Toxins

still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 13.

[0173] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 13.

[0174] In still another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a PAR2 ligand domain. In an aspect of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising SEQ ID NO: 24. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a naturally occurring PAR2 ligand domain variant, such as, *e.g.*, a PAR2 ligand domain isoform or a PAR2 ligand domain subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a naturally occurring PAR2 ligand domain variant of SEQ ID NO: 24, such as, *e.g.*, a PAR2 ligand domain isoform of SEQ ID NO: 24 or a PAR2 ligand domain subtype of SEQ ID NO: 24. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a non-naturally occurring PAR2 ligand domain variant, such as, *e.g.*, a conservative PAR2 ligand domain variant, a non-conservative PAR2 ligand domain variant or a PAR2 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a non-naturally occurring PAR2 ligand domain variant of SEQ ID NO: 24, such as, *e.g.*, a conservative PAR2 ligand domain variant of SEQ ID NO: 24, a non-conservative PAR2 ligand domain variant of SEQ ID NO: 24 or a PAR2 ligand domain peptidomimetic of SEQ ID NO: 24, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising SEQ ID NO: 24 or SEQ ID NO: 25.

[0175] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 24, at least 67%

Steward, L.E. *et al.*, Degradable Clostridial Toxins

amino acid identity with the SEQ ID NO: 24, or at least 83% amino acid identity with SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 24, at most 67% amino acid identity with the SEQ ID NO: 24, at most 83% amino acid identity with SEQ ID NO: 24.

**[0176]** In other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 24.

**[0177]** In other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 24.

**[0178]** In still another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a PAR3 ligand domain. In an aspect of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising SEQ ID NO: 26. In another aspect of this embodiment, a polynucleotide

Steward, L.E. *et al.*, Degradable Clostridial Toxins

molecule encodes a PAR3 ligand domain comprising a naturally occurring PAR3 ligand domain variant, such as, *e.g.*, a PAR3 ligand domain isoform or a PAR3 ligand domain subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a naturally occurring PAR3 ligand domain variant of SEQ ID NO: 26, such as, *e.g.*, a PAR3 ligand domain isoform of SEQ ID NO: 26 or a PAR3 ligand domain subtype of SEQ ID NO: 26. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a non-naturally occurring PAR3 ligand domain variant, such as, *e.g.*, a conservative PAR3 ligand domain variant, a non-conservative PAR3 ligand domain variant or a PAR3 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a non-naturally occurring PAR3 ligand domain variant of SEQ ID NO: 26, such as, *e.g.*, a conservative PAR3 ligand domain variant of SEQ ID NO: 26, a non-conservative PAR3 ligand domain variant of SEQ ID NO: 26 or a PAR3 ligand domain peptidomimetic of SEQ ID NO: 26, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising SEQ ID NO: 26 or SEQ ID NO: 27.

**[0179]** In other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 26, at least 67% amino acid identity with the SEQ ID NO: 26, or at least 83% amino acid identity with SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 26, at most 67% amino acid identity with the SEQ ID NO: 26, at most 83% amino acid identity with SEQ ID NO: 26.

**[0180]** In other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 26.



Steward, L.E. *et al.*, Degradable Clostridial Toxins

**[0181]** In other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 26.

**[0182]** In still another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a PAR4 ligand domain. In an aspect of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising SEQ ID NO: 28. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a naturally occurring PAR4 ligand domain variant, such as, *e.g.*, a PAR4 ligand domain isoform or a PAR4 ligand domain subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a naturally occurring PAR4 ligand domain variant of SEQ ID NO: 28, such as, *e.g.*, a PAR4 ligand domain isoform of SEQ ID NO: 28 or a PAR4 ligand domain subtype of SEQ ID NO: 28. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a non-naturally occurring PAR4 ligand domain variant, such as, *e.g.*, a conservative PAR4 ligand domain variant, a non-conservative PAR4 ligand domain variant or a PAR4 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a non-naturally occurring PAR4 ligand domain variant of SEQ ID NO: 28, such as, *e.g.*, a conservative PAR4 ligand domain variant of SEQ ID NO: 28, a non-conservative PAR4 ligand domain variant of SEQ ID NO: 28 or a PAR4 ligand domain peptidomimetic of SEQ ID NO: 28, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46 or SEQ ID NO: 47.

**[0183]** In other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 28, at least 67% amino acid identity with the SEQ ID NO: 28, or at least 83% amino acid identity with SEQ ID NO: 28. In

Steward, L.E. *et al.*, Degradable Clostridial Toxins

still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 28, at most 67% amino acid identity with the SEQ ID NO: 28, at most 83% amino acid identity with SEQ ID NO: 28.

[0184] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 28.

[0185] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 28.

[0186] In yet another embodiment, a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification can further comprise a polynucleotide molecule encoding a flexible region comprising a flexible spacer. In another embodiment, a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification can further comprise a polynucleotide

Steward, L.E. *et al.*, Degradable Clostridial Toxins

molecule encoding a flexible region comprising a plurality of flexible spacers in tandem. In aspects of this embodiment, a polynucleotide molecule encoding a flexible region can comprise in tandem, *e.g.*, at least 1 G-spacer, at least 2 G-spacers, at least 3 G-spacers, at least 4 G-spacers or at least 5 G-spacers. In other aspects of this embodiment, a polynucleotide molecule encoding a flexible region can comprise in tandem, *e.g.*, at most 1 G-spacer, at most 2 G-spacers, at most 3 G-spacers, at most 4 G-spacers or at most 5 G-spacers. In still other aspects of this embodiment, a polynucleotide molecule encoding a flexible region can comprise in tandem, *e.g.*, at least 1 A-spacer, at least 2 A-spacers, at least 3 A-spacers, at least 4 A-spacers or at least 5 A-spacers. In still other aspects of this embodiment, a polynucleotide molecule encoding a flexible region can comprise in tandem, *e.g.*, at most 1 A-spacer, at most 2 A-spacers, at most 3 A-spacers, at most 4 A-spacers or at most 5 A-spacers. In another aspect of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise a polynucleotide molecule encoding a flexible region comprising one or more copies of the same flexible spacers, one or more copies of different flexible-spacers region, or any combination thereof.

[0187] In yet another embodiment, a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification can further comprises a polynucleotide molecule encoding an epitope-binding region. In another embodiment, a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification can further comprises a polynucleotide molecule encoding a plurality of epitope-binding regions. In aspects of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise, *e.g.*, at least 1 polynucleotide molecule encoding an epitope-binding region, at least 2 polynucleotide molecules encoding epitope-binding regions, at least 3 polynucleotide molecules encoding epitope-binding regions, at least 4 polynucleotide molecules encoding epitope-binding regions or at least 5 polynucleotide molecules encoding epitope-binding regions. In other aspects of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise, *e.g.*, at most 1 polynucleotide molecule encoding an epitope-binding region, at most 2 polynucleotide molecules encoding epitope-binding regions, at most 3 polynucleotide molecules encoding epitope-binding regions, at most 4 polynucleotide molecules encoding epitope-binding regions or at most 5 polynucleotide molecules encoding epitope-binding regions. In another aspect of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise one or more copies of the same polynucleotide molecules encoding epitope-binding region, one or more copies of different polynucleotide molecules encoding epitope-binding region, or any combination thereof. The location of a polynucleotide molecule encoding an epitope-binding region can be in various positions, including, without limitation, at the amino terminus of a modified Clostridial toxin, within a modified Clostridial toxin, or at the carboxyl terminus of a modified Clostridial toxin.

[0188] In yet another embodiment, polynucleotide molecules encoding a modified Clostridial toxin disclosed in the present specification can further comprise a polynucleotide molecule encoding an exogenous protease cleavage site. In another embodiment, a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification can further comprises a plurality of

Steward, L.E. *et al.*, Degradable Clostridial Toxins

polynucleotide molecules encoding exogenous protease cleavage sites. In aspects of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise, *e.g.*, at least 1 polynucleotide molecule encoding an exogenous protease cleavage site, at least 2 polynucleotide molecules encoding exogenous protease cleavage sites, at least 3 polynucleotide molecules encoding exogenous protease cleavage sites, at least 4 polynucleotide molecules encoding exogenous protease cleavage sites or at least 5 polynucleotide molecules encoding exogenous protease cleavage sites. In other aspects of this embodiment, polynucleotide molecules encoding a modified Clostridial toxin can comprise, *e.g.*, at most 1 polynucleotide molecule encoding an exogenous protease cleavage site, at most 2 polynucleotide molecules encoding exogenous protease cleavage sites, at most 3 polynucleotide molecules encoding exogenous protease cleavage sites, at most 4 polynucleotide molecules encoding exogenous protease cleavage sites or at most 5 polynucleotide molecules encoding exogenous protease cleavage sites. In another aspect of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise one or more copies of the same exogenous protease cleavage site, one or more copies of different exogenous protease cleavage site, or any combination thereof.

[0189] In yet another embodiment, a polynucleotide molecule encoding an exogenous protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding peptide and a polynucleotide molecule encoding a modified Clostridial toxin. In other aspects of this embodiment, a polynucleotide molecule encoding a bovine enterokinase cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin, a polynucleotide molecule encoding a Tobacco Etch Virus protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin, a polynucleotide molecule encoding a SUMO/ULP-1 protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin, a polynucleotide molecule encoding a Thrombin protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin, or a polynucleotide molecule encoding a Coagulation Factor Xa protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the bovine enterokinase protease cleavage site of SEQ ID NO: 50 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the Tobacco Etch Virus protease cleavage site of SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In still other

Steward, L.E. *et al.*, Degradable Clostridial Toxins

aspects of the embodiment, a polynucleotide molecule encoding the Human Rhinovirus 3C protease cleavage site of SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 66 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In yet other aspects of the embodiment, a polynucleotide molecule encoding the SUMO/ULP-1 protease cleavage site of SEQ ID NO: 67 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In further other aspects of the embodiment, a polynucleotide molecule encoding the Thrombin protease cleavage site of SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81 or SEQ ID NO: 82 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the Coagulation Factor Xa protease cleavage site of SEQ ID NO: 83 or SEQ ID NO: 84 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin.

**[0190]** In yet another embodiment, a polynucleotide molecule encoding an exogenous protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In aspects of this embodiment, a polynucleotide molecule encoding a bovine enterokinase cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin, a polynucleotide molecule encoding a Tobacco Etch Virus protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin, a polynucleotide molecule encoding a Human Rhinovirus 3C protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin, a polynucleotide molecule encoding a SUMO/ULP-1 protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin, a polynucleotide molecule encoding a Thrombin protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin, or a polynucleotide molecule encoding a Coagulation Factor Xa protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the bovine enterokinase protease cleavage site of SEQ ID NO: 50 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the Tobacco Etch Virus protease cleavage site of SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In still other aspects of the embodiment, a polynucleotide molecule encoding the Human Rhinovirus 3C protease cleavage site of SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 66 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In yet other aspects of the

Steward, C.E. *et al.*, Degradable Clostridial Toxins

embodiment, a polynucleotide molecule encoding the SUMO/ULP-1 protease cleavage site of SEQ ID NO: 67 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In further other aspects of the embodiment, a polynucleotide molecule encoding the Thrombin protease cleavage site of SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81 or SEQ ID NO: 82 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the Coagulation Factor Xa protease cleavage site of SEQ ID NO: 83 or SEQ ID NO: 84 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin.

**[0191]** Another aspect of the present invention provides a method of producing a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain, such method comprising the step of expressing a polynucleotide molecule encoding a modified Clostridial toxin in a cell. Another aspect of the present invention provides a method of producing a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain, such method comprising the steps of introducing an expression construct comprising a polynucleotide molecule encoding a modified Clostridial toxin into a cell and expressing the expression construct in the cell.

**[0192]** The methods disclosed in the present specification include, in part, a Clostridial toxin. It is envisioned that any and all Clostridial toxins disclosed in the present specification can be produced using the methods disclosed in the present specification. Thus, aspects of this embodiment include producing, without limitation, naturally occurring Clostridial toxins, naturally occurring Clostridial toxins variants, such as, *e.g.*, Clostridial toxins isoforms and Clostridial toxins subtypes, non-naturally occurring Clostridial toxins variants, such as, *e.g.*, conservative Clostridial toxins variants, non-conservative Clostridial toxins variants and Clostridial toxins fragments thereof, or any combination thereof.

**[0193]** The methods disclosed in the present specification include, in part, a PAR binding domain. It is envisioned that any and all PAR binding domains disclosed in the present specification can be produced using the methods disclosed in the present specification. Thus, aspects of this embodiment include producing, without limitation, naturally occurring PAR binding domains, naturally occurring PAR binding domain variants, such as, *e.g.*, PAR binding domain isoforms and PAR binding domain subtypes, non-naturally occurring PAR binding domain variants, such as, *e.g.*, conservative PAR binding domain variants, non-conservative PAR binding domain variants and PAR binding domain fragments thereof, or any combination thereof.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

**[0194]** The methods disclosed in the present specification include, in part, a polynucleotide molecule. It is envisioned that any and all polynucleotide molecules disclosed in the present specification can be used. Thus, aspects of this embodiment include, without limitation, polynucleotide molecules encoding naturally occurring Clostridial toxins; polynucleotide molecules encoding naturally occurring Clostridial toxins variants, such as, *e.g.*, Clostridial toxins isoforms and Clostridial toxins subtypes; polynucleotide molecules encoding non-naturally occurring Clostridial toxins variants, such as, *e.g.*, conservative Clostridial toxins variants, non-conservative Clostridial toxins variants and Clostridial toxins fragments thereof, or any combination thereof.

**[0195]** The methods disclosed in the present specification include, in part, an expression construct. An expression construct comprises a polynucleotide molecule disclosed in the present specification operably-linked to an expression vector useful for expressing the polynucleotide molecule in a cell or cell-free extract. A wide variety of expression vectors can be employed for expressing a polynucleotide molecule encoding a modified Clostridial toxin, including, without limitation, a viral expression vector; a prokaryotic expression vector; eukaryotic expression vectors, such as, *e.g.*, a yeast expression vector, an insect expression vector and a mammalian expression vector; and a cell-free extract expression vector. It is further understood that expression vectors useful to practice aspects of these methods may include those which express a modified Clostridial toxin under control of a constitutive, tissue-specific, cell-specific or inducible promoter element, enhancer element or both. Non-limiting examples of expression vectors, along with well-established reagents and conditions for making and using an expression construct from such expression vectors are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; EMD Biosciences-Novagen, Madison, WI; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. The selection, making and use of an appropriate expression vector are routine procedures well within the scope of one skilled in the art and from the teachings herein.

**[0196]** Thus, aspects of this embodiment include, without limitation, a viral expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; a prokaryotic expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; a yeast expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; an insect expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; and a mammalian expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin. Other aspects of this embodiment include, without limitation, expression constructs suitable for expressing a modified Clostridial toxin disclosed in the present specification using a cell-free extract comprising a cell-free extract expression vector operably linked to a polynucleotide molecule encoding a modified Clostridial toxin. Other aspects of this embodiment include, without limitation, expression constructs comprising polynucleotide molecules comprising any one of SEQ ID NO: 109 through SEQ ID NO: 132 and SEQ ID NO: 136 through SEQ ID NO: 159. Other aspects of this

Steward, L.E. *et al.*, Degradable Clostridial Toxins

embodiment include, without limitation, expression constructs comprising polynucleotide molecules encoding a modified Clostridial toxin comprising any one of SEQ ID NO: 85 through SEQ ID NO: 108.

[0197] The methods disclosed in the present specification include, in part, a cell. It is envisioned that any and all cells can be used. Thus, aspects of this embodiment include, without limitation, prokaryotic cells including, without limitation, strains of aerobic, microaerophilic, capnophilic, facultative, anaerobic, gram-negative and gram-positive bacterial cells such as those derived from, *e.g.*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacteroides fragilis*, *Clostridia perfringens*, *Clostridia difficile*, *Caulobacter crescentus*, *Lactococcus lactis*, *Methylobacterium extorquens*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Pseudomonas fluorescens* and *Salmonella typhimurium*; and eukaryotic cells including, without limitation, yeast strains, such as, *e.g.*, those derived from *Pichia pastoris*, *Pichia methanolica*, *Pichia angusta*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*; insect cells and cell lines derived from insects, such as, *e.g.*, those derived from *Spodoptera frugiperda*, *Trichoplusia ni*, *Drosophila melanogaster* and *Manduca sexta*; and mammalian cells and cell lines derived from mammalian cells, such as, *e.g.*, those derived from mouse, rat, hamster, porcine, bovine, equine, primate and human. Cell lines may be obtained from the American Type Culture Collection (2004), at URL address [www.atcc.org](http://www.atcc.org); European Collection of Cell Cultures (2204), at URL address [www.ecacc.org.uk](http://www.ecacc.org.uk); and the German Collection of Microorganisms and Cell Cultures (2004), at URL address [www.dsmz.de](http://www.dsmz.de). Non-limiting examples of specific protocols for selecting, making and using an appropriate cell line are described in *e.g.*, INSECT CELL CULTURE ENGINEERING (Mattheus F. A. Goosen *et al.* eds., Marcel Dekker, 1993); INSECT CELL CULTURES: FUNDAMENTAL AND APPLIED ASPECTS (J. M. Vlak *et al.* eds., Kluwer Academic Publishers, 1996); Maureen A. Harrison & Ian F. Rae, GENERAL TECHNIQUES OF CELL CULTURE (Cambridge University Press, 1997); CELL AND TISSUE CULTURE: LABORATORY PROCEDURES (Alan Doyle *et al.* eds., John Wiley and Sons, 1998); R. Ian Freshney, CULTURE OF ANIMAL CELLS: A MANUAL OF BASIC TECHNIQUE (Wiley-Liss, 4<sup>th</sup> ed. 2000); ANIMAL CELL CULTURE: A PRACTICAL APPROACH (John R. W. Masters ed., Oxford University Press, 3<sup>rd</sup> ed. 2000); MOLECULAR CLONING A LABORATORY MANUAL, *supra*, (2001); BASIC CELL CULTURE: A PRACTICAL APPROACH (John M. Davis, Oxford Press, 2<sup>nd</sup> ed. 2002); and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, *supra*, (2004). These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0198] The methods disclosed in the present specification include, in part, introducing into a cell a polynucleotide molecule. A polynucleotide molecule introduced into a cell can be transiently or stably maintained by that cell. Stably-maintained polynucleotide molecules may be extra-chromosomal and replicate autonomously, or they may be integrated into the chromosomal material of the cell and replicate non-autonomously. It is envisioned that any and all methods for introducing a polynucleotide molecule disclosed in the present specification into a cell can be used. Methods useful for introducing a nucleic acid molecule into a cell include, without limitation, chemical-mediated transfection such as, *e.g.*, calcium phosphate-mediated, diethyl-aminoethyl (DEAE) dextran-mediated, lipid-mediated, polyethyleneimine (PEI)-mediated, polylysine-mediated and polybrene-mediated; physical-mediated transfection, such as,



Steward, L.E. *et al.*, Degradable Clostridial Toxins

*e.g.*, biolistic particle delivery, microinjection, protoplast fusion and electroporation; and viral-mediated transfection, such as, *e.g.*, retroviral-mediated transfection, see, *e.g.*, *Introducing Cloned Genes into Cultured Mammalian Cells*, pp. 16.1-16.62 (Sambrook & Russell, eds., *Molecular Cloning A Laboratory Manual*, Vol. 3, 3<sup>rd</sup> ed. 2001). One skilled in the art understands that selection of a specific method to introduce an expression construct into a cell will depend, in part, on whether the cell will transiently contain an expression construct or whether the cell will stably contain an expression construct. These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

**[0199]** In an aspect of this embodiment, a chemical-mediated method, termed transfection, is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. In chemical-mediated methods of transfection the chemical reagent forms a complex with the nucleic acid that facilitates its uptake into the cells. Such chemical reagents include, without limitation, calcium phosphate-mediated, see, *e.g.*, Martin Jordan & Florian Worm, *Transfection of adherent and suspended cells by calcium phosphate*, 33(2) *Methods* 136-143 (2004); diethyl-aminoethyl (DEAE) dextran-mediated, lipid-mediated, cationic polymer-mediated like polyethyleneimine (PEI)-mediated and polylysine-mediated and polybrene-mediated, see, *e.g.*, Chun Zhang et al., *Polyethylenimine strategies for plasmid delivery to brain-derived cells*, 33(2) *Methods* 144-150 (2004). Such chemical-mediated delivery systems can be prepared by standard methods and are commercially available, see, *e.g.*, CellPfect Transfection Kit (Amersham Biosciences, Piscataway, NJ); Mammalian Transfection Kit, Calcium phosphate and DEAE Dextran, (Stratagene, Inc., La Jolla, CA); Lipofectamine™ Transfection Reagent (Invitrogen, Inc., Carlsbad, CA); ExGen 500 Transfection kit (Fermentas, Inc., Hanover, MD), and SuperFect and Effectene Transfection Kits (Qiagen, Inc., Valencia, CA).

**[0200]** In another aspect of this embodiment, a physical-mediated method is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. Physical techniques include, without limitation, electroporation, biolistic and microinjection. Biolistics and microinjection techniques perforate the cell wall in order to introduce the nucleic acid molecule into the cell, see, *e.g.*, Jeike E. Biewenga et al., *Plasmid-mediated gene transfer in neurons using the biolistics technique*, 71(1) *J. Neurosci. Methods*. 67-75 (1997); and John O'Brien & Sarah C. R. Lummis, *Biolistic and diolistic transfection: using the gene gun to deliver DNA and lipophilic dyes into mammalian cells*, 33(2) *Methods* 121-125 (2004). Electroporation, also termed electroporabilization, uses brief, high-voltage, electrical pulses to create transient pores in the membrane through which the nucleic acid molecules enter and can be used effectively for stable and transient transfections of all cell types, see, *e.g.*, M. Golzio et al., *In vitro and in vivo electric field-mediated permeabilization, gene transfer, and expression*, 33(2) *Methods* 126-135 (2004); and Oliver Greschet al., *New non-viral method for gene transfer into primary cells*, 33(2) *Methods* 151-163 (2004).

**[0201]** In another aspect of this embodiment, a viral-mediated method, termed transduction, is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. In viral-mediated

Steward, L.E. *et al.*, Degradable Clostridial Toxins

methods of transient transduction, the process by which viral particles infect and replicate in a host cell has been manipulated in order to use this mechanism to introduce a nucleic acid molecule into the cell. Viral-mediated methods have been developed from a wide variety of viruses including, without limitation, retroviruses, adenoviruses, adeno-associated viruses, herpes simplex viruses, picornaviruses, alphaviruses and baculoviruses, see, *e.g.*, Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, 33(2) *Methods* 164-172 (2004); and Maurizio Federico, From lentiviruses to lentivirus vectors, 229 *Methods Mol. Biol.* 3-15 (2003); E. M. Poeschla, Non-primate lentiviral vectors, 5(5) *Curr. Opin. Mol. Ther.* 529-540 (2003); Karim Benihoud *et al.*, Adenovirus vectors for gene delivery, 10(5) *Curr. Opin. Biotechnol.* 440-447 (1999); H. Bueler, Adeno-associated viral vectors for gene transfer and gene therapy, 380(6) *Biol. Chem.* 613-622 (1999); Chooi M. Lai *et al.*, Adenovirus and adeno-associated virus vectors, 21(12) *DNA Cell Biol.* 895-913 (2002); Edward A. Burton *et al.*, Gene delivery using herpes simplex virus vectors, 21(12) *DNA Cell Biol.* 915-936 (2002); Paola Grandi *et al.*, Targeting HSV amplicon vectors, 33(2) *Methods* 179-186 (2004); Ilya Frolov *et al.*, Alphavirus-based expression vectors: strategies and applications, 93(21) *Proc. Natl. Acad. Sci. U. S. A.* 11371-11377 (1996); Markus U. Ehrenguber, Alphaviral gene transfer in neurobiology, 59(1) *Brain Res. Bull.* 13-22 (2002); Thomas A. Kost & J. Patrick Condreay, Recombinant baculoviruses as mammalian cell gene-delivery vectors, 20(4) *Trends Biotechnol.* 173-180 (2002); and A. Huser & C. Hofmann, Baculovirus vectors: novel mammalian cell gene-delivery vehicles and their applications, 3(1) *Am. J. Pharmacogenomics* 53-63 (2003).

**[0202]** Adenoviruses, which are non-enveloped, double-stranded DNA viruses, are often selected for mammalian cell transduction because adenoviruses handle relatively large polynucleotide molecules of about 36 kb, are produced at high titer, and can efficiently infect a wide variety of both dividing and non-dividing cells, see, *e.g.*, Wim T. J. M. C. Hermens *et al.*, Transient gene transfer to neurons and glia: analysis of adenoviral vector performance in the CNS and PNS, 71(1) *J. Neurosci. Methods* 85-98 (1997); and Hiroyuki Mizuguchi *et al.*, Approaches for generating recombinant adenovirus vectors, 52(3) *Adv. Drug Deliv. Rev.* 165-176 (2001). Transduction using adenoviral-based system do not support prolonged protein expression because the nucleic acid molecule is carried from an episome in the cell nucleus, rather than being integrated into the host cell chromosome. Adenoviral vector systems and specific protocols for how to use such vectors are disclosed in, *e.g.*, ViraPower™ Adenoviral Expression System (Invitrogen, Inc., Carlsbad, CA) and ViraPower™ Adenoviral Expression System Instruction Manual 25-0543 version A, Invitrogen, Inc., (Jul. 15, 2002); and AdEasy™ Adenoviral Vector System (Stratagene, Inc., La Jolla, CA) and AdEasy™ Adenoviral Vector System Instruction Manual 064004f, Stratagene, Inc..

**[0203]** Nucleic acid molecule delivery can also use single-stranded RNA retroviruses, such as, *e.g.*, oncoretroviruses and lentiviruses. Retroviral-mediated transduction often produce transduction efficiencies close to 100%, can easily control the proviral copy number by varying the multiplicity of infection (MOI), and can be used to either transiently or stably transduce cells, see, *e.g.*, Tiziana Tonini *et al.*, Transient production of retroviral- and lentiviral-based vectors for the transduction of Mammalian cells,

Steward, L.E. *et al.*, Degradable Clostridial Toxins

285 Methods Mol. Biol. 141-148 (2004); Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, 33(2) Methods 164-172 (2004); Félix Recillas-Targa, Gene transfer and expression in mammalian cell lines and transgenic animals, 267 Methods Mol. Biol. 417-433 (2004); and Roland Wolkowicz *et al.*, Lentiviral vectors for the delivery of DNA into mammalian cells, 246 Methods Mol. Biol. 391-411 (2004). Retroviral particles consist of an RNA genome packaged in a protein capsid, surrounded by a lipid envelope. The retrovirus infects a host cell by injecting its RNA into the cytoplasm along with the reverse transcriptase enzyme. The RNA template is then reverse transcribed into a linear, double stranded cDNA that replicates itself by integrating into the host cell genome. Viral particles are spread both vertically (from parent cell to daughter cells via the provirus) as well as horizontally (from cell to cell via virions). This replication strategy enables long-term persistent expression since the nucleic acid molecules of interest are stably integrated into a chromosome of the host cell, thereby enabling long-term expression of the protein. For instance, animal studies have shown that lentiviral vectors injected into a variety of tissues produced sustained protein expression for more than 1 year, see, *e.g.*, Luigi Naldini *et al.*, In vivo gene delivery and stable transduction of non-dividing cells by a lentiviral vector, 272(5259) Science 263-267 (1996). The Oncoretroviruses-derived vector systems, such as, *e.g.*, Moloney murine leukemia virus (MoMLV), are widely used and infect many different non-dividing cells. Lentiviruses can also infect many different cell types, including dividing and non-dividing cells and possess complex envelope proteins, which allows for highly specific cellular targeting.

[0204] Retroviral vectors and specific protocols for how to use such vectors are disclosed in, *e.g.*, U.S. Patent Nos. Manfred Gossen & Hermann Bujard, Tight control of gene expression in eukaryotic cells by tetracycline-responsive promoters, U.S. Patent No. 5,464,758 (Nov. 7, 1995) and Hermann Bujard & Manfred Gossen, Methods for regulating gene expression, U.S. Patent No. 5,814,618 (Sep. 29, 1998) David S. Hogness, Polynucleotides encoding insect steroid hormone receptor polypeptides and cells transformed with same, U.S. Patent No. 5,514,578 (May 7, 1996) and David S. Hogness, Polynucleotide encoding insect ecdysone receptor, U.S. Patent 6,245,531 (Jun. 12, 2001); Elisabetta Vegeto *et al.*, Progesterone receptor having C. terminal hormone binding domain truncations, U.S. Patent No. 5,364,791 (Nov. 15, 1994), Elisabetta Vegeto *et al.*, Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy, U.S. Patent No. 5,874,534 (Feb. 23, 1999) and Elisabetta Vegeto *et al.*, Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy, U.S. Patent No. 5,935,934 (Aug. 10, 1999). Furthermore, such viral delivery systems can be prepared by standard methods and are commercially available, see, *e.g.*, BD™ Tet-Off and Tet-On Gene Expression Systems (BD Biosciences-Clontech, Palo Alto, CA) and BD™ Tet-Off and Tet-On Gene Expression Systems User Manual, PT3001-1, BD Biosciences Clontech, (Mar. 14, 2003), GeneSwitch™ System (Invitrogen, Inc., Carlsbad, CA) and GeneSwitch™ System A Mifepristone-Regulated Expression System for Mammalian Cells version D, 25-0313, Invitrogen, Inc., (Nov. 4, 2002); ViraPower™ Lentiviral Expression System (Invitrogen, Inc., Carlsbad, CA) and ViraPower™ Lentiviral Expression System Instruction Manual 25-0501 version E, Invitrogen, Inc., (Dec. 8, 2003); and Complete Control® Retroviral

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Inducible Mammalian Expression System (Stratagene, La Jolla, CA) and Complete Control<sup>®</sup> Retroviral Inducible Mammalian Expression System Instruction Manual, 064005e.

[0205] The methods disclosed in the present specification include, in part, expressing a modified Clostridial toxin from a polynucleotide molecule. It is envisioned that any of a variety of expression systems may be useful for expressing a modified Clostridial toxin from a polynucleotide molecule disclosed in the present specification, including, without limitation, cell-based systems and cell-free expression systems. Cell-based systems include, without limitation, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems and mammalian expression systems. Cell-free systems include, without limitation, wheat germ extracts, rabbit reticulocyte extracts and *E. coli* extracts and generally are equivalent to the method disclosed herein. Expression of a polynucleotide molecule using an expression system can include any of a variety of characteristics including, without limitation, inducible expression, non-inducible expression, constitutive expression, viral-mediated expression, stably-integrated expression, and transient expression. Expression systems that include well-characterized vectors, reagents, conditions and cells are well-established and are readily available from commercial vendors that include, without limitation, Ambion, Inc. Austin, TX; BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc. Carlsbad, CA; QIAGEN, Inc., Valencia, CA; Roche Applied Science, Indianapolis, IN; and Stratagene, La Jolla, CA. Non-limiting examples on the selection and use of appropriate heterologous expression systems are described in *e.g.*, PROTEIN EXPRESSION. A PRACTICAL APPROACH (S. J. Higgins and B. David Hames eds., Oxford University Press, 1999); Joseph M. Fernandez & James P. Hoeffler, GENE EXPRESSION SYSTEMS. USING NATURE FOR THE ART OF EXPRESSION (Academic Press, 1999); and Meena Rai & Harish Padh, *Expression Systems for Production of Heterologous Proteins*, 80(9) CURRENT SCIENCE 1121-1128, (2001). These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0206] A variety of cell-based expression procedures are useful for expressing a modified Clostridial toxin encoded by polynucleotide molecule disclosed in the present specification. Examples included, without limitation, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems and mammalian expression systems. Viral expression systems include, without limitation, the ViraPower<sup>™</sup> Lentiviral (Invitrogen, Inc., Carlsbad, CA), the Adenoviral Expression Systems (Invitrogen, Inc., Carlsbad, CA), the AdEasy<sup>™</sup> XL Adenoviral Vector System (Stratagene, La Jolla, CA) and the ViraPort<sup>®</sup> Retroviral Gene Expression System (Stratagene, La Jolla, CA). Non-limiting examples of prokaryotic expression systems include the Champion<sup>™</sup> pET Expression System (EMD Biosciences-Novagen, Madison, WI), the TriEx<sup>™</sup> Bacterial Expression Systems (EMD Biosciences-Novagen, Madison, WI), the QIAexpress<sup>®</sup> Expression System (QIAGEN, Inc.), and the Affinity<sup>®</sup> Protein Expression and Purification System (Stratagene, La Jolla, CA). Yeast expression systems include, without limitation, the EasySelect<sup>™</sup> *Pichia* Expression Kit (Invitrogen, Inc., Carlsbad, CA), the YES-Echo<sup>™</sup> Expression Vector Kits (Invitrogen, Inc., Carlsbad, CA) and the

Steward, L.E. *et al.*, Degradable Clostridial Toxins

SpECTRA™ *S. pombe* Expression System (Invitrogen, Inc., Carlsbad, CA). Non-limiting examples of baculoviral expression systems include the BaculoDirect™ (Invitrogen, Inc., Carlsbad, CA), the Bac-to-Bac® (Invitrogen, Inc., Carlsbad, CA), and the BD BaculoGold™ (BD Biosciences-Pharmingen, San Diego, CA). Insect expression systems include, without limitation, the *Drosophila* Expression System (DES®) (Invitrogen, Inc., Carlsbad, CA), InsectSelect™ System (Invitrogen, Inc., Carlsbad, CA) and InsectDirect™ System (EMD Biosciences-Novagen, Madison, WI). Non-limiting examples of mammalian expression systems include the T-REx™ (Tetracycline-Regulated Expression) System (Invitrogen, Inc., Carlsbad, CA), the Flp-In™ T-REx™ System (Invitrogen, Inc., Carlsbad, CA), the pcDNA™ system (Invitrogen, Inc., Carlsbad, CA), the pSecTag2 system (Invitrogen, Inc., Carlsbad, CA), the Exchanger® System, InterPlay™ Mammalian TAP System (Stratagene, La Jolla, CA), Complete Control® Inducible Mammalian Expression System (Stratagene, La Jolla, CA) and LacSwitch® II Inducible Mammalian Expression System (Stratagene, La Jolla, CA).

[0207] Another procedure of expressing a modified Clostridial toxin encoded by polynucleotide molecule disclosed in the present specification employs a cell-free expression system such as, without limitation, prokaryotic extracts and eukaryotic extracts. Non-limiting examples of prokaryotic cell extracts include the RTS 100 *E. coli* HY Kit (Roche Applied Science, Indianapolis, IN), the ActivePro In Vitro Translation Kit (Ambion, Inc., Austin, TX), the EcoPro™ System (EMD Biosciences-Novagen, Madison, WI) and the Expressway™ Plus Expression System (Invitrogen, Inc., Carlsbad, CA). Eukaryotic cell extract include, without limitation, the RTS 100 Wheat Germ CEF Kit (Roche Applied Science, Indianapolis, IN), the TnT® Coupled Wheat Germ Extract Systems (Promega Corp., Madison, WI), the Wheat Germ IVT™ Kit (Ambion, Inc., Austin, TX), the Retic Lysate IVT™ Kit (Ambion, Inc., Austin, TX), the PROTEINscript® II System (Ambion, Inc., Austin, TX) and the TnT® Coupled Reticulocyte Lysate Systems (Promega Corp., Madison, WI).

## EXAMPLES

[0208] The following non-limiting examples are provided for illustrative purposes only in order to facilitate a more complete understanding of disclosed embodiments and are in no way intended to limit any of the embodiments disclosed in the present specification.

### Example 1

#### Construction of BoNT/A-ED-PAR1Tb

[0209] This example illustrates how to make a modified Clostridial toxin comprising a PAR binding domain located at the amino terminus of the light chain comprising the enzymatic domain.

[0210] A polynucleotide molecule (SEQ ID NO: 109) based on BoNT/A-ED-PAR1Tb (SEQ ID NO: 85) is synthesized using standard procedures (BlueHeron® Biotechnology, Bothell, WA). Oligonucleotides of 20

Steward, L.E. *et al.*, Degradable Clostridial Toxins

to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-ED-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

**[0211]** If desired, an expression optimized polynucleotide molecule (SEQ ID NO: 136) based on BoNT/A-ED-PAR1Tb (SEQ ID NO: 85) can be synthesized in order to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-ED-PAR1Tb can be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, *e.g.*, Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type E, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type A, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-ED-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). If so desired, optimization to a different organism, such as, *e.g.*, a yeast strain, an insect cell-line or a mammalian cell line, can be done, see, *e.g.*, Steward, *supra*, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); and Steward, *supra*, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005).

**[0212]** A similar cloning strategy is used to make pUCBHB1 cloning constructs comprising the polynucleotide molecule of SEQ ID NO: 110 or SEQ ID NO: 137 encoding BoNT/A-ED-PAR1Xa of SEQ ID NO: 86; the polynucleotide molecule of SEQ ID NO: 111 or SEQ ID NO: 138 encoding BoNT/A-ED-PAR2Tp of SEQ ID NO: 87; the polynucleotide molecule of SEQ ID NO: 112 or SEQ ID NO: 139 encoding BoNT/A-ED-PAR2Xa of SEQ ID NO: 88; the polynucleotide molecule of SEQ ID NO: 113 or SEQ ID NO: 140 encoding BoNT/A-ED-PAR3Tb of SEQ ID NO: 89; the polynucleotide molecule of SEQ ID NO: 114 or SEQ ID NO: 141 encoding BoNT/A-ED-PAR3Xa of SEQ ID NO: 90; the polynucleotide molecule of SEQ ID NO: 115 or SEQ ID NO: 142 encoding BoNT/A-ED-PAR4Tb of SEQ ID NO: 91; and the polynucleotide molecule of SEQ ID NO: 116 or SEQ ID NO: 143 encoding BoNT/A-ED-PAR4Xa of SEQ ID NO: 92. In addition, one skilled in the art can modify Clostridial toxins, such as, *e.g.*, BoNT/B,

Steward, L.E. *et al.*, Degradable Clostridial Toxins

BoNT/C1, BoNT/D, BoNT/E, BoNT/F, BoNT/G and TeNT, so that these toxins possess the PAR attributes of the modified BoNT/A described above and make them using similar cloning strategy.

**[0213]** To construct pET29/BoNT/A-ED-PAR1Tb, a pUCBHB1/BoNT/A-ED-PAR1Tb construct is digested with restriction endonucleases that 1) excise the insert comprising the open reading frame of SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb; and 2) enable this insert to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert is subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-ED-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5 $\alpha$  cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50  $\mu$ g/mL of Kanamycin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Kanamycin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pET29 expression construct comprising the polynucleotide molecule of SEQ ID NO: 136 encoding the BoNT/A-ED-PAR1Tb of SEQ ID NO: 85 operably-linked to a carboxyl terminal polyhistidine affinity binding peptide (FIG. 7).

**[0214]** A similar cloning strategy is used to make pET29 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 109 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85; SEQ ID NO: 110 or SEQ ID NO: 137 encoding BoNT/A-ED-PAR1Xa of SEQ ID NO: 86; the polynucleotide molecule of SEQ ID NO: 111 or SEQ ID NO: 138 encoding BoNT/A-ED-PAR2Tp of SEQ ID NO: 87; the polynucleotide molecule of SEQ ID NO: 112 or SEQ ID NO: 139 encoding BoNT/A-ED-PAR2Xa of SEQ ID NO: 88; the polynucleotide molecule of SEQ ID NO: 113 or SEQ ID NO: 140 encoding BoNT/A-ED-PAR3Tb of SEQ ID NO: 89; the polynucleotide molecule of SEQ ID NO: 114 or SEQ ID NO: 141 encoding BoNT/A-ED-PAR3Xa of SEQ ID NO: 90; the polynucleotide molecule of SEQ ID NO: 115 or SEQ ID NO: 142 encoding BoNT/A-ED-PAR4Tb of SEQ ID NO: 91; and the polynucleotide molecule of SEQ ID NO: 116 or SEQ ID NO: 143 encoding BoNT/A-ED-PAR4Xa of SEQ ID NO: 92.

## Example 2

### Construction of BoNT/A-TD-PAR1Tb

**[0215]** This example illustrates how to make a modified Clostridial toxin comprising a PAR binding domain located at the amino terminus of the heavy chain region comprising the translocation domain.

**[0216]** A polynucleotide molecule (SEQ ID NO: 117) based on BoNT/A-TD-PAR1Tb (SEQ ID NO: 93) is synthesized using standard procedures (BlueHeron® Biotechnology, Bothell, WA). Oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length

Steward, L.E. *et al.*, Degradable Clostridial Toxins

polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-TD-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0217] If desired, an expression optimized polynucleotide molecule (SEQ ID NO: 144) based on BoNT/A-TD-PAR1Tb (SEQ ID NO: 93) can be synthesized in order to improve expression in an *Escherichia coli* strain. The open reading frame comprising the polynucleotide molecule is optimized to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-TD-PAR1Tb can be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, *e.g.*, Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type E, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type A, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate BoNT/A-TD-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). Is so desired, optimization of the polynucleotide molecule encoding a BoNT/A-TD-PAR1Tb need not be performed, or optimization to a different organism, such as, *e.g.*, a yeast strain, an insect cell-line or a mammalian cell line, can be done instead, see, *e.g.*, Steward, *supra*, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); and Steward, *supra*, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005).

[0218] A similar cloning strategy is used to make pUCBHB1 cloning constructs comprising the polynucleotide molecule of SEQ ID NO: 118 or SEQ ID NO: 145 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 94; the polynucleotide molecule of SEQ ID NO: 119 or SEQ ID NO: 146 encoding BoNT/A-TD-PAR2Tp of SEQ ID NO: 95; the polynucleotide molecule of SEQ ID NO: 120 or SEQ ID NO: 147 encoding BoNT/A-TD-PAR2Xa of SEQ ID NO: 96; the polynucleotide molecule of SEQ ID NO: 121 or SEQ ID NO: 148 encoding BoNT/A-TD-PAR1Tb of SEQ ID NO: 97; the polynucleotide molecule of SEQ ID NO: 122 or SEQ ID NO: 149 encoding BoNT/A-TD-PAR3Xa of SEQ ID NO: 98; the polynucleotide molecule of SEQ ID NO: 123 or SEQ ID NO: 150 encoding BoNT/A-TD-PAR4Tb of SEQ ID NO: 99; and the polynucleotide molecule of SEQ ID NO: 124 or SEQ ID NO: 151 encoding BoNT/A-TD-PAR4Xa of SEQ ID NO: 100. In addition, one skilled in the art can modify Clostridial toxins, such as, *e.g.*, BoNT/B,



Steward, L.E. *et al.*, Degradable Clostridial Toxins

BoNT/C1, BoNT/D, BoNT/E, BoNT/F, BoNT/G and TeNT, so that these toxins possess the PAR attributes of the modified BoNT/A described above and make them using similar cloning strategy.

[0219] To construct pET29/BoNT/A-TD-PAR1Tb, a pUCBHB1/BoNT/A-TD-PAR1Tb construct is digested with restriction endonucleases that 1) excise the insert comprising the open reading frame of SEQ ID NO: 144 encoding BoNT/A-TD-PAR1Tb; and 2) enable this insert to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert is subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-TD-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5 $\alpha$  cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50  $\mu$ g/mL of Kanamycin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Kanamycin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pET29 expression construct comprising the polynucleotide molecule of SEQ ID NO: 144 encoding the BoNT/A-TD-PAR1Tb of SEQ ID NO: 93 operably-linked to a carboxyl terminal polyhistidine affinity binding peptide (FIG. 8).

[0220] A similar cloning strategy is used to make pET29 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 117 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 93; SEQ ID NO: 118 or SEQ ID NO: 145 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 94; the polynucleotide molecule of SEQ ID NO: 119 or SEQ ID NO: 146 encoding BoNT/A-TD-PAR2Tp of SEQ ID NO: 95; the polynucleotide molecule of SEQ ID NO: 120 or SEQ ID NO: 147 encoding BoNT/A-TD-PAR2Xa of SEQ ID NO: 96; the polynucleotide molecule of SEQ ID NO: 121 or SEQ ID NO: 148 encoding BoNT/A-TD-PAR1Tb of SEQ ID NO: 97; the polynucleotide molecule of SEQ ID NO: 122 or SEQ ID NO: 149 encoding BoNT/A-TD-PAR3Xa of SEQ ID NO: 98; the polynucleotide molecule of SEQ ID NO: 123 or SEQ ID NO: 150 encoding BoNT/A-TD-PAR4Tb of SEQ ID NO: 99; and the polynucleotide molecule of SEQ ID NO: 124 or SEQ ID NO: 151 encoding BoNT/A-TD-PAR4Xa of SEQ ID NO: 100.

### Example 3

#### Construction of BoNT/A-BD-PAR1Tb

[0221] This example illustrates how to make a modified Clostridial toxin comprising a PAR binding domain located at the amino terminus of the heavy chain region comprising the binding domain.

[0222] A polynucleotide molecule (SEQ ID NO: 125) based on BoNT/A-BD-PAR1Tb (SEQ ID NO: 101) is synthesized using standard procedures (BlueHeron® Biotechnology, Bothell, WA). Oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the

Steward, L.E. *et al.*, Degradable Clostridial Toxins

full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-BD-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0223] If desired, an expression optimized polynucleotide molecule (SEQ ID NO: 152) based on BoNT/A-BD-PAR1Tb (SEQ ID NO: 101) can be synthesized in order to improve expression in an *Escherichia coli* strain. The open reading frame comprising the polynucleotide molecule is optimized to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-BD-PAR1Tb can be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, *e.g.*, Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type E, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type A, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate BoNT/A-BD-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). If so desired, optimization of the polynucleotide molecule encoding a BoNT/A-BD-PAR1Tb need not be performed, or optimization to a different organism, such as, *e.g.*, a yeast strain, an insect cell-line or a mammalian cell line, can be done instead, see, *e.g.*, Steward, *supra*, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); and Steward, *supra*, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005).

[0224] A similar cloning strategy is used to make pUCBHB1 cloning constructs comprising the polynucleotide molecule of SEQ ID NO: 126 or SEQ ID NO: 153 encoding BoNT/A-BD-PAR1Xa of SEQ ID NO: 102; the polynucleotide molecule of SEQ ID NO: 127 or SEQ ID NO: 154 encoding BoNT/A-BD-PAR2Tp of SEQ ID NO: 103; the polynucleotide molecule of SEQ ID NO: 128 or SEQ ID NO: 155 encoding BoNT/A-BD-PAR2Xa of SEQ ID NO: 104; the polynucleotide molecule of SEQ ID NO: 129 or SEQ ID NO: 156 encoding BoNT/A-BD-PAR3Tb of SEQ ID NO: 105; the polynucleotide molecule of SEQ ID NO: 130 or SEQ ID NO: 157 encoding BoNT/A-BD-PAR3Xa of SEQ ID NO: 106; the polynucleotide molecule of SEQ ID NO: 131 or SEQ ID NO: 158 encoding BoNT/A-BD-PAR4Tb of SEQ ID NO: 107; and the polynucleotide molecule of SEQ ID NO: 132 or SEQ ID NO: 159 encoding BoNT/A-BD-PAR4Xa of SEQ ID NO: 108. In addition, one skilled in the art can modify Clostridial toxins, such as, *e.g.*, BoNT/B,

Steward, L.E. *et al.*, Degradable Clostridial Toxins

BoNT/C1, BoNT/D, BoNT/E, BoNT/F, BoNT/G and TeNT, so that these toxins possess the PAR attributes of the modified BoNT/A described above and make them using similar cloning strategy.

**[0225]** To construct pET29/BoNT/A-BD-PAR1Tb, a pUCBHB1/BoNT/A-BD-PAR1Tb construct is digested with restriction endonucleases that 1) excise the insert comprising the open reading frame of SEQ ID NO: 152 encoding BoNT/A-BD-PAR1Tb; and 2) enable this insert to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert is subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-BD-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5 $\alpha$  cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50  $\mu$ g/mL of Kanamycin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Kanamycin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pET29 expression construct comprising the polynucleotide molecule of SEQ ID NO: 152 encoding the BoNT/A-BD-PAR1Tb of SEQ ID NO: 101 operably-linked to a carboxyl terminal polyhistidine affinity binding peptide (FIG. 9).

**[0226]** A similar cloning strategy is used to make pET29 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 125 encoding BoNT/A-BD-PAR1Tb of SEQ ID NO: 101; the polynucleotide molecule of SEQ ID NO: 126 or SEQ ID NO: 153 encoding BoNT/A-BD-PAR1Xa of SEQ ID NO: 102; the polynucleotide molecule of SEQ ID NO: 127 or SEQ ID NO: 154 encoding BoNT/A-BD-PAR2Tp of SEQ ID NO: 103; the polynucleotide molecule of SEQ ID NO: 128 or SEQ ID NO: 155 encoding BoNT/A-BD-PAR2Xa of SEQ ID NO: 104; the polynucleotide molecule of SEQ ID NO: 129 or SEQ ID NO: 156 encoding BoNT/A-BD-PAR3Tb of SEQ ID NO: 105; the polynucleotide molecule of SEQ ID NO: 130 or SEQ ID NO: 157 encoding BoNT/A-BD-PAR3Xa of SEQ ID NO: 106; the polynucleotide molecule of SEQ ID NO: 131 or SEQ ID NO: 158 encoding BoNT/A-BD-PAR4Tb of SEQ ID NO: 107; and the polynucleotide molecule of SEQ ID NO: 132 or SEQ ID NO: 159 encoding BoNT/A-BD-PAR4Xa of SEQ ID NO: 108.

## Example 4

## Expression of Modified Clostridial Toxins in a Bacterial Cell

**[0227]** The following example illustrates a procedure useful for expressing any of the modified Clostridial toxins disclosed in the present specification in a bacterial cell.

**[0228]** An expression construct, such as, *e.g.*, pET29/BoNT/A-ED-PAR1Tb, pET29/BoNT/A-TD-PAR1Tb or pET29/BoNT/A-BD-PAR1Tb, see, *e.g.*, Examples 1, 2 and 3, is introduced into chemically competent *E. coli* BL21 (DE3) cells (Invitrogen, Inc, Carlsbad, CA) using a heat-shock transformation protocol. The

Steward, L.E. *et al.*, Degradable Clostridial Toxins

heat-shock reaction is plated onto 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 µg/mL of Kanamycin and is placed in a 37 °C incubator for overnight growth. Kanamycin-resistant colonies of transformed *E. coli* containing the expression construct, such as, *e.g.*, pET29/BoNT/A-ED-PAR1Tb, pET29/BoNT/A-TD-PAR1Tb or pET29/BoNT/A-BD-PAR1Tb, are used to inoculate a baffled flask containing 3.0 mL of PA-0.5G media containing 50 µg/mL of Kanamycin which is then placed in a 37 °C incubator, shaking at 250 rpm, for overnight growth. The resulting overnight starter culture is in turn used to inoculate a 3 L baffled flask containing ZYP-5052 autoinducing media containing 50 µg/mL of Kanamycin at a dilution of 1:1000. Culture volumes ranged from about 600 mL (20% flask volume) to about 750 mL (25% flask volume). These cultures are grown in a 37 °C incubator shaking at 250 rpm for approximately 5.5 hours and are then transferred to a 16 °C incubator shaking at 250 rpm for overnight expression. Cells are harvested by centrifugation (4,000 rpm at 4 °C for 20-30 minutes) and are used immediately, or stored dry at -80 °C until needed.

## Example 5

## Purification and Quantification of Modified Clostridial Toxins

[0229] The following example illustrates methods useful for purification and quantification of any modified Clostridial toxins disclosed in the present specification.

[0230] For immobilized metal affinity chromatography (IMAC) protein purification, *E. coli* BL21 (DE3) cell pellets used to express a modified Clostridial toxin, as described in Example 4, are resuspended in Column Binding Buffer (25 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 10 mM imidazole; 2x Protease Inhibitor Cocktail Set III (EMD Biosciences-Calbiochem, San Diego CA); 5 units/mL of Benzonase (EMD Biosciences-Novagen, Madison, WI); 0.1% (v/v) Triton-X<sup>®</sup> 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol), and then are transferred to a cold Oakridge centrifuge tube. The cell suspension is sonicated on ice (10-12 pulses of 10 seconds at 40% amplitude with 60 seconds cooling intervals on a Branson Digital Sonifier) in order to lyse the cells and then is centrifuged (16,000 rpm at 4 °C for 20 minutes) to clarify the lysate. An immobilized metal affinity chromatography column is prepared using a 20 mL Econo-Pac column support (Bio-Rad Laboratories, Hercules, CA) packed with 2.5-5.0 mL of TALON<sup>™</sup> SuperFlow Co<sup>2+</sup> affinity resin (BD Biosciences-Clontech, Palo Alto, CA), which is then equilibrated by rinsing with 5 column volumes of deionized, distilled water, followed by 5 column volumes of Column Binding Buffer. The clarified lysate is applied slowly to the equilibrated column by gravity flow (approximately 0.25-0.3 mL/minute). The column is then washed with 5 column volumes of Column Wash Buffer (*N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 10 mM imidazole; 0.1% (v/v) Triton-X<sup>®</sup> 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol). The Clostridial toxin is eluted with 20-30 mL of Column Elution Buffer (25 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 500 mM imidazole; 0.1% (v/v) Triton-X<sup>®</sup> 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol) and is collected in approximately twelve 1 mL fractions. The amount of Clostridial toxin

Steward, L.E. *et al.*, Degradable Clostridial Toxins

contained in each elution fraction is determined by a Bradford dye assay. In this procedure, 20  $\mu$ L aliquots of each 1.0 mL fraction is combined with 200  $\mu$ L of Bio-Rad Protein Reagent (Bio-Rad Laboratories, Hercules, CA), diluted 1 to 4 with deionized, distilled water, and then the intensity of the colorimetric signal is measured using a spectrophotometer. The five fractions with the strongest signal are considered the elution peak and are combined together. Total protein yield is determined by estimating the total protein concentration of the pooled peak elution fractions using bovine gamma globulin as a standard (Bio-Rad Laboratories, Hercules, CA).

**[0231]** For purification of a modified Clostridial toxin using a FPLC desalting column, a HiPrep™ 26/10 size exclusion column (Amersham Biosciences, Piscataway, NJ) is pre-equilibrated with 80 mL of 4 °C Column Buffer (50 mM sodium phosphate, pH 6.5). After the column is equilibrated, a Clostridial toxin sample is applied to the size exclusion column with an isocratic mobile phase of 4 °C Column Buffer and at a flow rate of 10 mL/minute using a BioLogic DuoFlow chromatography system (Bio-Rad Laboratories, Hercules, CA). The desalted modified Clostridial toxin sample is collected as a single fraction of approximately 7-12 mL.

**[0232]** For purification of a modified Clostridial toxin using a FPLC ion exchange column, a Clostridial toxin sample that has been desalted following elution from an IMAC column is applied to a 1 mL Q1™ anion exchange column (Bio-Rad Laboratories, Hercules, CA) using a BioLogic DuoFlow chromatography system (Bio-Rad Laboratories, Hercules, CA). The sample is applied to the column in 4 °C Column Buffer (50 mM sodium phosphate, pH 6.5) and is eluted by linear gradient with 4 °C Elution Buffer (50 mM sodium phosphate, 1 M sodium chloride, pH 6.5) as follows: step 1, 5.0 mL of 5% Elution Buffer at a flow rate of 1 mL/minute; step 2, 20.0 mL of 5-30% Elution Buffer at a flow rate of 1 mL/minute; step 3, 2.0 mL of 50% Elution Buffer at a flow rate of 1.0 mL/minute; step 4, 4.0 mL of 100% Elution Buffer at a flow rate of 1.0 mL/minute; and step 5, 5.0 mL of 0% Elution Buffer at a flow rate of 1.0 mL/minute. Elution of Clostridial toxin from the column is monitored at 280, 260, and 214 nm, and peaks absorbing above a minimum threshold (0.01 au) at 280 nm are collected. Most of the Clostridial toxin will elute at a sodium chloride concentration of approximately 100 to 200 mM. Average total yields of Clostridial toxin will be determined by a Bradford assay.

**[0233]** Expression of a modified Clostridial toxin is analyzed by polyacrylamide gel electrophoresis. Samples purified using the procedure described above are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and are separated by MOPS polyacrylamide gel electrophoresis using NuPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under denaturing, reducing conditions. Gels are stained with SYPRO® Ruby (Bio-Rad Laboratories, Hercules, CA) and the separated polypeptides are imaged using a Fluor-S MAX Multimager (Bio-Rad Laboratories, Hercules, CA) for quantification of Clostridial toxin expression levels. The size and amount of the Clostridial toxin is determined by comparison to MagicMark™ protein molecular weight standards (Invitrogen, Inc, Carlsbad, CA).

**[0234]** Expression of modified Clostridial toxin is also analyzed by Western blot analysis. Protein samples purified using the procedure described above are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and are separated by MOPS polyacrylamide gel electrophoresis using NuPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under denaturing, reducing conditions. Separated polypeptides are transferred from the gel onto polyvinylidene fluoride (PVDF) membranes (Invitrogen, Inc, Carlsbad, CA) by Western blotting using a Trans-Blot® SD semi-dry electrophoretic transfer cell apparatus (Bio-Rad Laboratories, Hercules, CA). PVDF membranes are blocked by incubating at room temperature for 2 hours in a solution containing 25 mM Tris-Buffered Saline (25 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl)(pH 7.4), 137 mM sodium chloride, 2.7 mM potassium chloride), 0.1% TWEEN-20®, polyoxyethylene (20) sorbitan monolaureate, 2% bovine serum albumin, 5% nonfat dry milk. Blocked membranes are incubated at 4 °C for overnight in Tris-Buffered Saline TWEEN-20® (25 mM Tris-Buffered Saline, 0.1% TWEEN-20®, polyoxyethylene (20) sorbitan monolaureate) containing appropriate primary antibodies as a probe. Primary antibody probed blots are washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20®. Washed membranes are incubated at room temperature for 2 hours in Tris-Buffered Saline TWEEN-20® containing an appropriate immunoglobulin G antibody conjugated to horseradish peroxidase as a secondary antibody. Secondary antibody-probed blots are washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20®. Signal detection of the labeled Clostridial toxin are visualized using the ECL Plus™ Western Blot Detection System (Amersham Biosciences, Piscataway, NJ) and are imaged with a Typhoon 9410 Variable Mode Imager (Amersham Biosciences, Piscataway, NJ) for quantification of modified Clostridial toxin expression levels.

#### Example 6

##### Expression of Modified Clostridial Toxins in a Yeast Cell

**[0235]** The following example illustrates a procedure useful for expressing any of the modified Clostridial toxins disclosed in the present specification in a yeast cell.

**[0236]** To construct a suitable yeast expression construct encoding a modified Clostridial toxin, restriction endonuclease sites suitable for cloning an operably linked polynucleotide molecule into a pPIC A vector (Invitrogen, Inc, Carlsbad, CA) are incorporated into the 5'- and 3' ends of the polynucleotide molecule SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85. This polynucleotide molecule is synthesized and a pUCBHB1/BoNT/A-ED-PAR1Tb construct is obtained as described in Example 1. This construct is digested with restriction enzymes that 1) excise the insert containing the open reading frame of SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb; and 2) enable this insert to be operably-linked to a pPIC A vector. This insert is subcloned using a T4 DNA ligase procedure into a pPIC A vector that is digested with appropriate restriction endonucleases to yield pPIC A/BoNT/A-ED-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5α cells (Invitrogen, Inc,

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Carlsbad, CA) using a heat shock method, plated on 1.5% low salt Luria-Bertani agar plates (pH 7.5) containing 25 µg/mL of Zeocin™, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Zeocin™ resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pPIC A expression construct comprising the polynucleotide molecule of SEQ ID NO: 136 encoding the BoNT/A-ED-PAR1Tb of SEQ ID NO: 85 operably-linked to a carboxyl-terminal c-myc and polyhistidine binding peptides (FIG. 10).

**[0237]** A similar cloning strategy is used to make pPIC A expression constructs comprising the polynucleotide molecule of SEQ ID NO: 109 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85; SEQ ID NO: 110 or SEQ ID NO: 137 encoding BoNT/A-ED-PAR1Xa of SEQ ID NO: 86; the polynucleotide molecule of SEQ ID NO: 111 or SEQ ID NO: 138 encoding BoNT/A-ED-PAR2Tp of SEQ ID NO: 87; the polynucleotide molecule of SEQ ID NO: 112 or SEQ ID NO: 139 encoding BoNT/A-ED-PAR2Xa of SEQ ID NO: 88; the polynucleotide molecule of SEQ ID NO: 113 or SEQ ID NO: 140 encoding BoNT/A-ED-PAR3Tb of SEQ ID NO: 89; the polynucleotide molecule of SEQ ID NO: 114 or SEQ ID NO: 141 encoding BoNT/A-ED-PAR3Xa of SEQ ID NO: 90; the polynucleotide molecule of SEQ ID NO: 115 or SEQ ID NO: 142 encoding BoNT/A-ED-PAR4Tb of SEQ ID NO: 91; and the polynucleotide molecule of SEQ ID NO: 116 or SEQ ID NO: 143 encoding BoNT/A-ED-PAR4Xa of SEQ ID NO: 92.

**[0238]** A similar cloning strategy is used to make pPIC A expression constructs comprising the polynucleotide molecule of SEQ ID NO: 117 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 93; SEQ ID NO: 118 or SEQ ID NO: 145 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 94; the polynucleotide molecule of SEQ ID NO: 119 or SEQ ID NO: 146 encoding BoNT/A-TD-PAR2Tp of SEQ ID NO: 95; the polynucleotide molecule of SEQ ID NO: 120 or SEQ ID NO: 147 encoding BoNT/A-TD-PAR2Xa of SEQ ID NO: 96; the polynucleotide molecule of SEQ ID NO: 121 or SEQ ID NO: 148 encoding BoNT/A-TD-PAR1Tb of SEQ ID NO: 97; the polynucleotide molecule of SEQ ID NO: 122 or SEQ ID NO: 149 encoding BoNT/A-TD-PAR3Xa of SEQ ID NO: 98; the polynucleotide molecule of SEQ ID NO: 123 or SEQ ID NO: 150 encoding BoNT/A-TD-PAR4Tb of SEQ ID NO: 99; and the polynucleotide molecule of SEQ ID NO: 124 or SEQ ID NO: 151 encoding BoNT/A-TD-PAR4Xa of SEQ ID NO: 100.

**[0239]** A similar cloning strategy is used to make pPIC A expression constructs comprising the polynucleotide molecule of SEQ ID NO: 125 encoding BoNT/A-BD-PAR1Tb of SEQ ID NO: 101; the polynucleotide molecule of SEQ ID NO: 126 or SEQ ID NO: 153 encoding BoNT/A-BD-PAR1Xa of SEQ ID NO: 102; the polynucleotide molecule of SEQ ID NO: 127 or SEQ ID NO: 154 encoding BoNT/A-BD-PAR2Tp of SEQ ID NO: 103; the polynucleotide molecule of SEQ ID NO: 128 or SEQ ID NO: 155 encoding BoNT/A-BD-PAR2Xa of SEQ ID NO: 104; the polynucleotide molecule of SEQ ID NO: 129 or SEQ ID NO: 156 encoding BoNT/A-BD-PAR3Tb of SEQ ID NO: 105; the polynucleotide molecule of SEQ ID NO: 130 or SEQ ID NO: 157 encoding BoNT/A-BD-PAR3Xa of SEQ ID NO: 106; the polynucleotide

Steward, L.E. *et al.*, Degradable Clostridial Toxins

molecule of SEQ ID NO: 131 or SEQ ID NO: 158 encoding BoNT/A-BD-PAR4Tb of SEQ ID NO: 107; and the polynucleotide molecule of SEQ ID NO: 132 or SEQ ID NO: 159 encoding BoNT/A-BD-PAR4Xa of SEQ ID NO: 108.

[0240] To construct a yeast cell line expressing a modified Clostridial toxin, pPICZ A/BoNT/A-ED-PAR1Tb is digested with a suitable restriction endonuclease (*i.e.*, *SacI*, *PmeI* or *BstXI*) and the resulting linearized expression construct is transformed into an appropriate *P. pastoris* Mut<sup>S</sup> strain KM71H using an electroporation method. The transformation mixture is plated on 1.5% YPDS agar plates (pH 7.5) containing 100 µg/mL of Zeocin<sup>TM</sup> and placed in a 28-30 °C incubator for 1-3 days of growth. Selection of transformants integrating the pPICZ A/BoNT/A-ED-PAR1Tb at the 5' AOX1 locus is determined by colony resistance to Zeocin<sup>TM</sup>. A similar strategy is used to make a cell line containing a pPICZ A expression construct containing SEQ ID NO: 2 used as a control for expression levels. Cell lines integrating a pPICZ A/BoNT/A-ED-PAR1Tb construct is tested for BoNT/A-ED-PAR1Tb expression using a small-scale expression test. Isolated colonies from test cell lines that have integrated pPICZ A/BoNT/A-ED-PAR1Tb are used to inoculate 1.0 L baffled flasks containing 100 mL of MGYH media and grown at about 28-30 °C in a shaker incubator (250 rpm) until the culture reaches an OD<sub>600</sub>=2-6 (approximately 16-18 hours). Cells are harvested by centrifugation (3,000x *g* at 22 °C for 5 minutes). To induce expression, the cell pellet is resuspended in 15 mL of MMH media and 100% methanol is added to a final concentration of 0.5%. Cultures are grown at about 28-30 °C in a shaker incubator (250 rpm) for six days. Additional 100% methanol is added to the culture every 24 hours to a final concentration of 0.5%. A 1.0 mL test aliquot is taken from the culture every 24 hours starting at time zero and ending at time 144 hours. Cells are harvested from the aliquots by microcentrifugation to pellet the cells and lysed using three freeze-thaw rounds consisting of -80 °C for 5 minutes, then 37 °C for 5 minutes. Lysis samples are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and expression from established cell lines is measured by Western blot analysis (as described in Example 5) using either anti-BoNT/A, anti-myc or anti-His antibodies in order to identify lines expressing BoNT/A-ED-PAR1Tb. The *P. pastoris* Mut<sup>S</sup> KM71H cell line showing the highest expression level of BoNT/A-ED-PAR1Tb is selected for large-scale expression using commercial fermentation procedures. Procedures for large-scale expression are as outlined above except the culture volume is approximately 2.5 L MGYH media grown in a 5 L BioFlo 3000 fermentor and concentrations of all reagents will be proportionally increased for this volume.

[0241] BoNT/A-ED-PAR1Tb is purified using the IMAC procedure, as described in Example 5. Expression from each culture is evaluated by a Bradford dye assay, polyacrylamide gel electrophoresis and Western blot analysis (as described in Example 5) in order to determine whether the amounts of BoNT/A-ED-PAR1Tb produced.

#### Example 7

##### Expression of Modified Clostridial Toxins in an Insect Cell



Steward, L.E. *et al.*, Degradable Clostridial Toxins

[0242] The following example illustrates a procedure useful for expressing any of the modified Clostridial toxins disclosed in the present specification in an insect cell.

[0243] To construct suitable an insect expression construct encoding a modified Clostridial toxin, restriction endonuclease sites suitable for cloning an operably linked polynucleotide molecule into a pBACgus3 vector (EMD Biosciences-Novagen, Madison, WI) are incorporated into the 5'- and 3' ends of the polynucleotide molecule SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85. This polynucleotide molecule is synthesized and a pUCBHB1/BoNT/A-ED-PAR1Tb construct is obtained as described in Example 1. This construct is digested with restriction enzymes that 1) excise the insert containing the open reading frame of SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb; and 2) enable this insert to be operably-linked to a pBACgus3 vector. This insert is subcloned using a T4 DNA ligase procedure into a pBACgus3 vector that is digested with appropriate restriction endonucleases to yield pBACgus3/BoNT/A-ED-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5 $\alpha$  cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 100  $\mu$ g/mL of Ampicillin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Ampicillin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pBACgus3 expression construct comprising the polynucleotide molecule of SEQ ID NO: 136 encoding the BoNT/A-ED-PAR1Tb of SEQ ID NO: 85 operably linked to an amino-terminal gp64 signal peptide and a carboxyl-terminal, Thrombin cleavable, polyhistidine affinity binding peptide (FIG. 11).

[0244] A similar cloning strategy is used to make pBACgus3 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 109 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85; SEQ ID NO: 110 or SEQ ID NO: 137 encoding BoNT/A-ED-PAR1Xa of SEQ ID NO: 86; the polynucleotide molecule of SEQ ID NO: 111 or SEQ ID NO: 138 encoding BoNT/A-ED-PAR2Tp of SEQ ID NO: 87; the polynucleotide molecule of SEQ ID NO: 112 or SEQ ID NO: 139 encoding BoNT/A-ED-PAR2Xa of SEQ ID NO: 88; the polynucleotide molecule of SEQ ID NO: 113 or SEQ ID NO: 140 encoding BoNT/A-ED-PAR3Tb of SEQ ID NO: 89; the polynucleotide molecule of SEQ ID NO: 114 or SEQ ID NO: 141 encoding BoNT/A-ED-PAR3Xa of SEQ ID NO: 90; the polynucleotide molecule of SEQ ID NO: 115 or SEQ ID NO: 142 encoding BoNT/A-ED-PAR4Tb of SEQ ID NO: 91; and the polynucleotide molecule of SEQ ID NO: 116 or SEQ ID NO: 143 encoding BoNT/A-ED-PAR4Xa of SEQ ID NO: 92.

[0245] A similar cloning strategy is used to make pBACgus3 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 117 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 93; SEQ ID NO: 118 or SEQ ID NO: 145 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 94; the polynucleotide molecule of SEQ ID NO: 119 or SEQ ID NO: 146 encoding BoNT/A-TD-PAR2Tp of SEQ ID NO: 95; the polynucleotide molecule of SEQ ID NO: 120 or SEQ ID NO: 147 encoding BoNT/A-TD-PAR2Xa of SEQ

Steward, L.E. *et al.*, Degradable Clostridial Toxins

ID NO: 96; the polynucleotide molecule of SEQ ID NO: 121 or SEQ ID NO: 148 encoding BoNT/A-TD-PAR1Tb of SEQ ID NO: 97; the polynucleotide molecule of SEQ ID NO: 122 or SEQ ID NO: 149 encoding BoNT/A-TD-PAR3Xa of SEQ ID NO: 98; the polynucleotide molecule of SEQ ID NO: 123 or SEQ ID NO: 150 encoding BoNT/A-TD-PAR4Tb of SEQ ID NO: 99; and the polynucleotide molecule of SEQ ID NO: 124 or SEQ ID NO: 151 encoding BoNT/A-TD-PAR4Xa of SEQ ID NO: 100.

**[0246]** A similar cloning strategy is used to make pBACgus3 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 125 encoding BoNT/A-BD-PAR1Tb of SEQ ID NO: 101; the polynucleotide molecule of SEQ ID NO: 126 or SEQ ID NO: 153 encoding BoNT/A-BD-PAR1Xa of SEQ ID NO: 102; the polynucleotide molecule of SEQ ID NO: 127 or SEQ ID NO: 154 encoding BoNT/A-BD-PAR2Tp of SEQ ID NO: 103; the polynucleotide molecule of SEQ ID NO: 128 or SEQ ID NO: 155 encoding BoNT/A-BD-PAR2Xa of SEQ ID NO: 104; the polynucleotide molecule of SEQ ID NO: 129 or SEQ ID NO: 156 encoding BoNT/A-BD-PAR3Tb of SEQ ID NO: 105; the polynucleotide molecule of SEQ ID NO: 130 or SEQ ID NO: 157 encoding BoNT/A-BD-PAR3Xa of SEQ ID NO: 106; the polynucleotide molecule of SEQ ID NO: 131 or SEQ ID NO: 158 encoding BoNT/A-BD-PAR4Tb of SEQ ID NO: 107; and the polynucleotide molecule of SEQ ID NO: 132 or SEQ ID NO: 159 encoding BoNT/A-BD-PAR4Xa of SEQ ID NO: 108.

**[0247]** To express a modified Clostridial toxin using a baculoviral expression system, about  $2.5 \times 10^6$  Sf9 cells are plated in four 60 mm culture dishes containing 2 mL of BacVector<sup>®</sup> Insect media (EMD Biosciences-Novagen, Madison, WI) and incubated for approximately 20 minutes in a 28 °C incubator. For each transfection, a 50 µL transfection solution is prepared in a 6 mL polystyrene tube by adding 25 µL of BacVector<sup>®</sup> Insect media containing 100 ng of a pBACgus3 construct encoding a modified Clostridial toxin, such as, *e.g.*, pBACgus3/BoNT/A-ED-PAR1Tb, and 500 ng TlowE transfer plasmid to 25 µL of diluted Insect GeneJuice<sup>®</sup> containing 5 µL Insect GeneJuice<sup>®</sup> (EMD Biosciences-Novagen, Madison, WI) and 20 µL nuclease-free water and this solution is incubated for approximately 15 minutes. After the 15 minute incubation, add 450 µL BacVector<sup>®</sup> media to the transfection solution and mix gently. Using this stock transfection solution as the 1/10 dilution make additional transfection solutions of 1/50, 1/250 and 1/1250 dilutions. Add 100 µL of a transfection solution to the Sf9 cells from one of the four 60 mm culture dishes, twice washed with antibiotic-free, serum-free BacVector<sup>®</sup> Insect media and incubate at 22 °C. After one hour, add 6 mL of 1% BacPlaque agarose-BacVector<sup>®</sup> Insect media containing 5% bovine serum albumin. After the agarose is solidified, add 2 mL BacVector<sup>®</sup> Insect media containing 5% bovine serum albumin to the transfected cells and transfer the cells to a 28 °C incubator for 3-5 days until plaques are visible. After 3-5 days post-transfection, plaques in the monolayer will be stained for β-glucuronidase reporter gene activity to test for the presence of recombinant virus plaques containing pBACgus3/BoNT/A-ED-PAR1Tb by incubating the washed monolayer with 2 mL of BacVector<sup>®</sup> Insect media containing 30 µL of 20 mg/mL X-Gluc Solution (EMD Biosciences-Novagen, Madison, WI) for approximately 2 hours in a 28 °C incubator.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

[0248] After identifying candidate recombinant virus plaques, several candidate virus plaques are eluted and plaque purified. To elute a recombinant virus, transfer a plug containing a recombinant virus plaque with a sterile Pasteur pipet to 1 mL BacVector® Insect media (EMD Biosciences-Novagen, Madison, WI) in a sterile screw-cap vial. Incubate the vial for approximately 2 hours at 22 °C or for approximately 16 hours at 4 °C. For each recombinant virus plaque,  $2.5 \times 10^5$  Sf9 cells are plated in 35 mm culture dishes containing 2 mL of BacVector® Insect media (EMD Biosciences-Novagen, Madison, WI) and incubated for approximately 20 minutes in a 28 °C incubator. Remove the media and add 200 µL of eluted recombinant virus. After one hour, add 2 mL of 1% BacPlaque agarose-BacVector® Insect media containing 5% bovine serum albumin. After the agarose is solidified, add 1 mL BacVector® Insect media containing 5% bovine serum albumin to the transfected cells and transfer the cells to a 28 °C incubator for 3-5 days until plaques are visible. After 3-5 days post-transfection, plaques in the monolayer will be stained for  $\beta$ -glucuronidase reporter gene activity to test for the presence of recombinant virus plaques containing pBACgus3/BoNT/A-ED-PAR1Tb by incubating the washed monolayer with 2 mL of BacVector® Insect media containing 30 µL of 20 mg/mL X-Gluc Solution (EMD Biosciences-Novagen, Madison, WI) for approximately 2 hours in a 28 °C incubator.

[0249] To prepare a seed stock of virus, elute a recombinant virus by transferring a plug containing a recombinant virus plaque with a sterile Pasteur pipet to 1 mL BacVector® Insect media (EMD Biosciences-Novagen, Madison, WI) in a sterile screw-cap vial. Incubate the vial for approximately 16 hours at 4 °C. Approximately  $5 \times 10^5$  Sf9 cells are plated in T-25 flask containing 5 mL of BacVector® Insect media (EMD Biosciences-Novagen, Madison, WI) and are incubated for approximately 20 minutes in a 28 °C incubator. Remove the media and add 300 µL of eluted recombinant virus. After one hour, add 5 mL BacVector® Insect media containing 5% bovine serum albumin to the transfected cells and transfer the cells to a 28 °C incubator for 3-5 days until the majority of cells become unattached and unhealthy. The virus is harvested by transferring the media to 15 mL snap-cap tubes and centrifuging tubes at 1000x g for 5 minutes to remove debris. The clarified supernatant is transferred to fresh 15 mL snap-cap tubes and are stored at 4 °C.

[0250] To prepare a high titer stock of virus, approximately  $2 \times 10^7$  Sf9 cells are plated in T-75 flask containing 10 mL of BacVector® Insect media (EMD Biosciences-Novagen, Madison, WI) and are incubated for approximately 20 minutes in a 28 °C incubator. Remove the media and add 500 µL of virus seed stock. After one hour, add 10 mL BacVector® Insect media containing 5% bovine serum albumin to the transfected cells and transfer the cells to a 28 °C incubator for 3-5 days until the majority of cells become unattached and unhealthy. The virus is harvested by transferring the media to 15 mL snap-cap tubes and centrifuging tubes at 1000x g for 5 minutes to remove debris. The clarified supernatant is transferred to fresh 15 mL snap-cap tubes and are stored at 4 °C. High titer virus stocks should contain approximately  $2 \times 10^8$  to  $3 \times 10^9$  pfu of baculovirus.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

[0251] To express gp64-BoNT/A-ED-PAR1Tb using a baculoviral expression system, about  $1.25 \times 10^8$  Sf9 cells are seeded in a 1 L flask containing 250 mL of BacVector<sup>®</sup> Insect media and are grown in an orbital shaker (150 rpm) to a cell density of approximately  $5 \times 10^8$ . The culture is inoculated with approximately  $2.5 \times 10^9$  of high titer stock recombinant baculovirus and incubated for approximately 48 hours in a 28 °C orbital shaker (150 rpm). Media is harvested by transferring the media to tubes and centrifuging tubes at 500x *g* for 5 minutes to remove debris. Media samples are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and expression is measured by Western blot analysis (as described in Example 5) using either anti-BoNT/A or anti-His antibodies in order to identify baculoviral stocks expressing BoNT/A-ED-PAR1Tb.

[0252] BoNT/A-ED-PAR1Tb is purified using the IMAC procedure, as described in Example 5. Expression from each culture is evaluated by a Bradford dye assay, polyacrylamide gel electrophoresis and Western blot analysis (as described in Example 5) in order to determine whether the amounts of BoNT/A-ED-PAR1Tb produced.

#### Example 8

##### Expression of Modified Clostridial Toxins in a Mammalian Cell

[0253] The following example illustrates a procedure useful for expressing any of the modified Clostridial toxins disclosed in the present specification in a mammalian cell.

[0254] To construct a suitable mammalian expression construct encoding a modified Clostridial toxin, restriction endonuclease sites suitable for cloning an operably linked polynucleotide molecule into a pSecTag2 vector (Invitrogen, Inc, Carlsbad, CA) are incorporated into the 5'- and 3' ends of the polynucleotide molecule SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85. This polynucleotide molecule is synthesized and a pUCBHB1/BoNT/A-ED-PAR1Tb construct is obtained as described in Example 1. This construct is digested with restriction enzymes that 1) excise the insert containing the open reading frame of SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb; and 2) enable this insert to be operably-linked to a pSecTag2 vector. This insert is subcloned using a T4 DNA ligase procedure into a pSecTag2 vector that is digested with appropriate restriction endonucleases to yield pSecTag2/ BoNT/A-ED-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 100 µg/mL of Ampicillin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Ampicillin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pSecTag2 expression construct comprising the polynucleotide molecule of SEQ ID NO: 136 encoding the BoNT/A-ED-PAR1Tb of SEQ ID NO: 85 operably-linked to a carboxyl-terminal c-myc and polyhistidine binding peptides (FIG. 12).

Steward, L.E. *et al.*, Degradable Clostridial Toxins

[0255] A similar cloning strategy is used to make pSecTag2 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 109 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85; SEQ ID NO: 110 or SEQ ID NO: 137 encoding BoNT/A-ED-PAR1Xa of SEQ ID NO: 86; the polynucleotide molecule of SEQ ID NO: 111 or SEQ ID NO: 138 encoding BoNT/A-ED-PAR2Tp of SEQ ID NO: 87; the polynucleotide molecule of SEQ ID NO: 112 or SEQ ID NO: 139 encoding BoNT/A-ED-PAR2Xa of SEQ ID NO: 88; the polynucleotide molecule of SEQ ID NO: 113 or SEQ ID NO: 140 encoding BoNT/A-ED-PAR3Tb of SEQ ID NO: 89; the polynucleotide molecule of SEQ ID NO: 114 or SEQ ID NO: 141 encoding BoNT/A-ED-PAR3Xa of SEQ ID NO: 90; the polynucleotide molecule of SEQ ID NO: 115 or SEQ ID NO: 142 encoding BoNT/A-ED-PAR4Tb of SEQ ID NO: 91; and the polynucleotide molecule of SEQ ID NO: 116 or SEQ ID NO: 143 encoding BoNT/A-ED-PAR4Xa of SEQ ID NO: 92.

[0256] A similar cloning strategy is used to make pSecTag2 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 117 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 93; SEQ ID NO: 118 or SEQ ID NO: 145 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 94; the polynucleotide molecule of SEQ ID NO: 119 or SEQ ID NO: 146 encoding BoNT/A-TD-PAR2Tp of SEQ ID NO: 95; the polynucleotide molecule of SEQ ID NO: 120 or SEQ ID NO: 147 encoding BoNT/A-TD-PAR2Xa of SEQ ID NO: 96; the polynucleotide molecule of SEQ ID NO: 121 or SEQ ID NO: 148 encoding BoNT/A-TD-PAR1Tb of SEQ ID NO: 97; the polynucleotide molecule of SEQ ID NO: 122 or SEQ ID NO: 149 encoding BoNT/A-TD-PAR3Xa of SEQ ID NO: 98; the polynucleotide molecule of SEQ ID NO: 123 or SEQ ID NO: 150 encoding BoNT/A-TD-PAR4Tb of SEQ ID NO: 99; and the polynucleotide molecule of SEQ ID NO: 124 or SEQ ID NO: 151 encoding BoNT/A-TD-PAR4Xa of SEQ ID NO: 100.

[0257] A similar cloning strategy is used to make pSecTag2 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 125 encoding BoNT/A-BD-PAR1Tb of SEQ ID NO: 101; the polynucleotide molecule of SEQ ID NO: 126 or SEQ ID NO: 153 encoding BoNT/A-BD-PAR1Xa of SEQ ID NO: 102; the polynucleotide molecule of SEQ ID NO: 127 or SEQ ID NO: 154 encoding BoNT/A-BD-PAR2Tp of SEQ ID NO: 103; the polynucleotide molecule of SEQ ID NO: 128 or SEQ ID NO: 155 encoding BoNT/A-BD-PAR2Xa of SEQ ID NO: 104; the polynucleotide molecule of SEQ ID NO: 129 or SEQ ID NO: 156 encoding BoNT/A-BD-PAR3Tb of SEQ ID NO: 105; the polynucleotide molecule of SEQ ID NO: 130 or SEQ ID NO: 157 encoding BoNT/A-BD-PAR3Xa of SEQ ID NO: 106; the polynucleotide molecule of SEQ ID NO: 131 or SEQ ID NO: 158 encoding BoNT/A-BD-PAR4Tb of SEQ ID NO: 107; and the polynucleotide molecule of SEQ ID NO: 132 or SEQ ID NO: 159 encoding BoNT/A-BD-PAR4Xa of SEQ ID NO: 108.

[0258] To transiently express modified Clostridial toxin in a cell line, about  $1.5 \times 10^5$  SH-SY5Y cells are plated in a 35 mm tissue culture dish containing 3 mL of complete Dulbecco's Modified Eagle Media (DMEM), supplemented with 10% fetal bovine serum (FBS), 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad,

Steward, L.E. *et al.*, Degradable Clostridial Toxins

CA), and grown in a 37 °C incubator under 5% carbon dioxide until cells reach a density of about  $5 \times 10^5$  cells/ml (6-16 hours). A 500  $\mu$ L transfection solution is prepared by adding 250  $\mu$ L of OPTI-MEM Reduced Serum Medium containing 15  $\mu$ L of LipofectAmine 2000 (Invitrogen, Carlsbad, CA) incubated at room temperature for 5 minutes to 250  $\mu$ L of OPTI-MEM Reduced Serum Medium containing 5  $\mu$ g of a pSecTag2 expression construct encoding a modified Clostridial toxin, such as, *e.g.*, pSecTag2/BoNT/A-ED-PAR1Tb. This transfection is incubated at room temperature for approximately 20 minutes. The complete, supplemented DMEM media is replaced with 2 mL of OPTI-MEM Reduced Serum Medium and the 500  $\mu$ L transfection solution is added to the SH-SY5Y cells and the cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 6 to 18 hours. Transfection media is replaced with 3 mL of fresh complete, supplemented DMEM and the cells are incubated in a 37 °C incubator under 5% carbon dioxide for 48 hours. Both media and cells are collected for expression analysis of BoNT/A-ED-PAR1Tb. Media is harvested by transferring the media to 15 mL snap-cap tubes and centrifuging tubes at 500x *g* for 5 minutes to remove debris. Cells are harvested by rinsing cells once with 3.0 mL of 100 mM phosphate-buffered saline, pH 7.4 and lysing cells with a buffer containing 62.6 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl), pH 6.8 and 2% sodium lauryl sulfate (SDS). Both media and cell samples are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and expression is measured by Western blot analysis (as described in Example 5) using either anti-BoNT/A, anti-c-myc or anti-His antibodies in order to identify pSecTag2 constructs expressing BoNT/A-ED-PAR1Tb. A similar procedure can be used to transiently express a pSecTag2 construct encoding any of the modified Clostridial toxin of SEQ ID NO: 86 to SEQ ID NO: 108.

**[0259]** To generate a stably-integrated cell line expressing a modified Clostridial toxin, approximately  $1.5 \times 10^5$  SH-SY5Y cells are plated in a 35 mm tissue culture dish containing 3 mL of complete DMEM, supplemented with 10% FBS, 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad, CA), and grown in a 37 °C incubator under 5% carbon dioxide until cells reach a density of about  $5 \times 10^5$  cells/ml (6-16 hours). A 500  $\mu$ L transfection solution is prepared by adding 250  $\mu$ L of OPTI-MEM Reduced Serum Medium containing 15  $\mu$ L of LipofectAmine 2000 (Invitrogen, Carlsbad, CA) incubated at room temperature for 5 minutes to 250  $\mu$ L of OPTI-MEM Reduced Serum Medium containing 5  $\mu$ g of a pSecTag2 expression construct encoding a modified Clostridial toxin, such as, *e.g.*, pSecTag2/BoNT/A-ED-PAR1Tb. This transfection solution is incubated at room temperature for approximately 20 minutes. The complete, supplemented DMEM media is replaced with 2 mL of OPTI-MEM Reduced Serum Medium and the 500  $\mu$ L transfection solution is added to the SH-SY5Y cells and the cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 6 to 18 hours. Transfection media is replaced with 3 mL of fresh complete, supplemented DMEM and cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 48 hours. Media is replaced with 3 mL of fresh complete DMEM, containing approximately 5  $\mu$ g/mL of Zeocin™, 10% FBS, 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad, CA). Cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 3-4 weeks, with old media being replaced with fresh

Steward, L.E. *et al.*, Degradable Clostridial Toxins

Zeocin™-selective, complete, supplemented DMEM every 4 to 5 days. Once Zeocin™-resistant colonies are established, resistant clones are replated to new 35 mm culture plates containing fresh complete DMEM, supplemented with approximately 5 µg/mL of Zeocin™, 10% FBS, 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad, CA), until these cells reach a density of 6 to 20x10<sup>5</sup> cells/mL. To test for expression of BoNT/A-ED-PAR1Tb from SH-SY5Y cell lines that have stably-integrated a pSecTag2/BoNT/A-ED-PAR1Tb, approximately 1.5x10<sup>5</sup> SH-SY5Y cells from each cell line are plated in a 35 mm tissue culture dish containing 3 mL of Zeocin™-selective, complete, supplemented DMEM and grown in a 37 °C incubator under 5% carbon dioxide until cells reach a density of about 5x10<sup>5</sup> cells/ml (6-16 hours). Media is replaced with 3 mL of fresh Zeocin™-selective, complete, supplemented DMEM and cells are incubated in a 37 °C incubator under 5% carbon dioxide for 48 hours. Both media and cells are collected for expression analysis of BoNT/A-c-myc-His. Media is harvested by transferring the media to 15 mL snap-cap tubes and centrifuging tubes at 500x *g* for 5 minutes to remove debris. Cells are harvest by rinsing cells once with 3.0 mL of 100 mM phosphate-buffered saline, pH 7.4 and lysing cells with a buffer containing 62.6 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl), pH 6.8 and 2% sodium lauryl sulfate (SDS). Both media and cell samples are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and expression is measured by Western blot analysis (as described in Example 5) using either anti-BoNT/A, anti-c-myc or anti-His antibodies in order to identify SH-SY5Y cell lines expressing BoNT/A-ED-PAR1Tb. The established SH-SY5Y cell line showing the highest expression level of BoNT/A-ED-PAR1Tb is selected for large-scale expression using 3 L flasks. Procedures for large-scale expression are as outlined above except the starting volume is approximately 800-1000 mL of complete DMEM and concentrations of all reagents are proportionally increased for this volume. A similar procedure can be used to stably express a pSecTag2 construct encoding any of the modified Clostridial toxin of SEQ ID NO: 86 to SEQ ID NO: 108.

[0260] BoNT/A-ED-PAR1Tb is purified using the IMAC procedure, as described in Example 5. Expression from each culture is evaluated by a Bradford dye assay, polyacrylamide gel electrophoresis and Western blot analysis (as described in Example 5) in order to determine whether the amounts of BoNT/A-ED-PAR1Tb produced.

[0261] Although aspects of the present invention have been described with reference to the disclosed embodiments, one skilled in the art will readily appreciate that the specific examples disclosed are only illustrative of these aspects and in no way limit the present invention. Various modifications can be made without departing from the spirit of the present invention.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

What is claimed:

1. A modified Clostridial toxin comprising:
  - a) a PAR ligand domain;
  - b) a Clostridial toxin enzymatic domain;
  - c) a Clostridial toxin translocation domain; and
  - d) a Clostridial toxin binding domain.
2. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain is operationally-linked to the amino terminus of the Clostridial toxin enzymatic domain.
3. The modified Clostridial toxin according to Claim 2, wherein the modified Clostridial toxin comprises an amino to carboxyl single polypeptide linear order comprising the PAR ligand domain, the Clostridial toxin enzymatic domain, the Clostridial toxin translocation domain and the Clostridial toxin binding domain.
4. The modified Clostridial toxin according to Claim 2, wherein the modified Clostridial toxin comprises an amino to carboxyl single polypeptide linear order comprising the PAR ligand domain, the Clostridial toxin enzymatic domain, the Clostridial toxin binding domain and the Clostridial toxin translocation domain.
5. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain is operationally-linked to the amino terminus of the Clostridial toxin translocation domain.
6. The modified Clostridial toxin according to Claim 5, wherein the modified Clostridial toxin comprises an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin binding domain, the Clostridial toxin enzymatic domain, the PAR ligand domain and the Clostridial toxin translocation domain.
7. The modified Clostridial toxin according to Claim 5, wherein the modified Clostridial toxin comprises an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin enzymatic domain, the PAR ligand domain, the Clostridial toxin translocation domain and the Clostridial toxin binding domain.
8. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain is operationally-linked to the amino terminus of the Clostridial toxin binding domain.
9. The modified Clostridial toxin according to Claim 8, wherein the modified Clostridial toxin comprises an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin enzymatic domain, the PAR ligand domain, the Clostridial toxin binding domain and the Clostridial toxin translocation domain.
10. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin further comprises a protease cleavage site; wherein cleavage of the protease cleavage site unmasks the PAR ligand domain.
11. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain comprises a PAR1 ligand domain.
12. The modified Clostridial toxin according to Claim 11, wherein the PAR1 ligand domain comprises SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 or SEQ ID NO: 133.
13. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain comprises a PAR2 ligand domain.



- Steward, L.E. *et al.*, Degradable Clostridial Toxins

14. The modified Clostridial toxin according to Claim 13, wherein the PAR2 ligand domain comprises SEQ ID NO: 24 or SEQ ID NO: 25.
15. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain comprises a PAR3 ligand domain.
16. The modified Clostridial toxin according to Claim 15, wherein the PAR3 ligand domain comprises SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 134.
17. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain comprises a PAR4 ligand domain.
18. The modified Clostridial toxin according to Claim 17, wherein the PAR4 ligand domain comprises SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 135 or SEQ ID NO: 160.
19. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin is a modified Botulinum toxin comprising a PAR ligand domain, a Botulinum toxin enzymatic domain, a Botulinum toxin translocation domain and a Botulinum toxin binding domain.
20. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/A comprising a PAR ligand domain, a BoNT/A enzymatic domain, a BoNT/A translocation domain and a BoNT/A binding domain.
21. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/B comprising a PAR ligand domain, a BoNT/B enzymatic domain, a BoNT/B translocation domain and a BoNT/B binding domain.
22. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/C1 comprising a PAR ligand domain, a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain and a BoNT/C1 binding domain.
23. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/D comprising a PAR ligand domain, a BoNT/D enzymatic domain, a BoNT/D translocation domain and a BoNT/D binding domain.
24. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/E comprising a PAR ligand domain, a BoNT/E enzymatic domain, a BoNT/E translocation domain and a BoNT/E binding domain.
25. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/F comprising a PAR ligand domain, a BoNT/F enzymatic domain, a BoNT/F translocation domain and a BoNT/F binding domain.
26. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/G comprising a PAR ligand domain, a BoNT/G enzymatic domain, a BoNT/G translocation domain and a BoNT/G binding domain.
27. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin is a modified Tetanus toxin comprising a PAR ligand domain, a Tetanus toxin enzymatic domain, a Tetanus toxin translocation domain and a Tetanus toxin binding domain.
28. A polynucleotide molecule encoding a modified Clostridial toxin, the polynucleotide molecule comprising:
  - a) polynucleotide molecule encoding a PAR ligand domain;
  - b) polynucleotide molecule encoding a Clostridial toxin enzymatic domain;

Steward, L.E. *et al.*, Degradable Clostridial Toxins

- c) polynucleotide molecule encoding a Clostridial toxin translocation domain; and
  - d) polynucleotide molecule encoding a Clostridial toxin binding domain.
29. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encodes a polypeptide comprising the PAR ligand domain operationally-linked to the amino terminus of the Clostridial toxin enzymatic domain.
30. The polynucleotide molecule according to Claim 29, wherein the polynucleotide molecule encodes a modified Clostridial toxin comprising an amino to carboxyl single polypeptide linear order comprising the PAR ligand domain, the Clostridial toxin enzymatic domain, the Clostridial toxin translocation domain and the Clostridial toxin binding domain.
31. The polynucleotide molecule according to Claim 29, wherein the polynucleotide molecule encodes a modified Clostridial toxin comprising an amino to carboxyl single polypeptide linear order comprising the PAR ligand domain, the Clostridial toxin enzymatic domain, the Clostridial toxin binding domain and the Clostridial toxin translocation domain.
32. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encodes a polypeptide comprising the PAR ligand domain operationally-linked to the amino terminus of the Clostridial toxin translocation domain.
33. The polynucleotide molecule according to Claim 32, wherein the polynucleotide molecule encodes a modified Clostridial toxin comprising an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin binding domain, the Clostridial toxin enzymatic domain, the PAR ligand domain and the Clostridial toxin translocation domain.
34. The polynucleotide molecule according to Claim 32, wherein the polynucleotide molecule encodes a modified Clostridial toxin comprising an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin enzymatic domain, the PAR ligand domain, the Clostridial toxin translocation domain and the Clostridial toxin binding domain.
35. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encodes a polypeptide comprising the PAR ligand domain operationally-linked to the amino terminus of the Clostridial toxin binding domain.
36. The polynucleotide molecule according to Claim 35, wherein the polynucleotide molecule encodes a modified Clostridial toxin comprising an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin enzymatic domain, the PAR ligand domain, the Clostridial toxin binding domain and the Clostridial toxin translocation domain.
37. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule further encodes a protease cleavage site; wherein cleavage of the protease cleavage site un.masks the PAR ligand domain.
38. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encoding the PAR ligand domain comprises a PAR1 ligand domain.
39. The polynucleotide molecule according to Claim 38, wherein the polynucleotide molecule encodes the PAR1 ligand domain comprising SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 or SEQ ID NO: 133.
40. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encoding the PAR ligand domain comprises a PAR2 ligand domain.
41. The polynucleotide molecule according to Claim 40, wherein the polynucleotide molecule encodes the PAR2 ligand domain comprises SEQ ID NO: 24 or SEQ ID NO: 25.

Steward, L.E. *et al.*, Degradable Clostridial Toxins

42. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encoding the PAR ligand domain comprises a PAR3 ligand domain.
43. The polynucleotide molecule according to Claim 42, wherein the polynucleotide molecule encodes the PAR3 ligand domain comprises SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 134.
44. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encoding the PAR ligand domain comprises a PAR4 ligand domain.
45. The polynucleotide molecule according to Claim 44, wherein the polynucleotide molecule encodes the PAR4 ligand domain comprises SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 135 or SEQ ID NO: 160.
46. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encoding the modified Clostridial toxin comprises a polynucleotide molecule encoding a modified Botulinum toxin comprising a PAR ligand domain, a Botulinum toxin enzymatic domain, a Botulinum toxin translocation domain and a Botulinum toxin binding domain.
47. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/A comprising a PAR ligand domain, a BoNT/A enzymatic domain, a BoNT/A translocation domain and a BoNT/A binding domain.
48. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/B comprising a PAR ligand domain, a BoNT/B enzymatic domain, a BoNT/B translocation domain and a BoNT/B binding domain.
49. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/C1 comprising a PAR ligand domain, a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain and a BoNT/C1 binding domain.
50. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/D comprising a PAR ligand domain, a BoNT/D enzymatic domain, a BoNT/D translocation domain and a BoNT/D binding domain.
51. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/E comprising a PAR ligand domain, a BoNT/E enzymatic domain, a BoNT/E translocation domain and a BoNT/E binding domain.
52. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/F comprising a PAR ligand domain, a BoNT/F enzymatic domain, a BoNT/F translocation domain and a BoNT/F binding domain.
53. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/G comprising a PAR ligand domain, a BoNT/G enzymatic domain, a BoNT/G translocation domain and a BoNT/G binding domain.
54. The modified Clostridial toxin according to Claim 28, wherein the polynucleotide molecule encoding the modified Clostridial toxin comprises a polynucleotide molecule encoding a modified Tetanus toxin

Steward, L.E. *et al.*, Degradable Clostridial Toxins

comprising a PAR ligand domain, a Tetanus toxin enzymatic domain, a Tetanus toxin translocation domain and a Tetanus toxin binding domain.

55. A method of producing a modified Clostridial toxin comprising the step of expressing a modified Clostridial toxin encoded by a polynucleotide molecule in a cell, wherein the modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain.
56. A methods of producing a modified Clostridial toxin comprising the steps of:
  - a. introducing into a cell a polynucleotide molecule encoding a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; and
  - b. expressing the modified Clostridial toxin encoded by the polynucleotide molecule.
57. A modified Clostridial toxin comprising:
  - a) a PAR ligand domain;
  - b) a Clostridial toxin enzymatic domain;
  - c) a Clostridial toxin translocation domain; and
  - d) a non-Clostridial toxin binding domain.
58. The modified Clostridial toxin according to Claim 57, wherein the non-Clostridial toxin binding domain is selected from the group consisting of a Nerve growth factor (NGF), a Leukemia inhibitory factor (LIF), a Basic fibroblast growth factor (bFGF), a Brain-derived neurotrophic factor (BDNF), a Neurotrophin-3 (NT-3), a Hydra head activator peptide (HHAP), a Transforming growth factor 1 (TGF-1), a Transforming growth factor 2 (TGF-2), a Transforming growth factor 3(TGF-3), an Epidermal growth factor (EGF) or a Ciliary neurotrophic factor (CNTF).
59. The modified Clostridial toxin according to Claim 57, wherein the non-Clostridial toxin binding domain is selected from the group consisting of a Tumor necrosis factor (TNF-), an Interleukin-1 (IL-1), an Interleukin-1 (IL-1) or an Interleukin-8 (IL-8).
60. The modified Clostridial toxin according to Claim 57, wherein the non-Clostridial toxin binding domain is selected from the group consisting of a Bradykinin, a Dynorphin, a  $\beta$ -endorphin, an Etorphine, an Endomorphin-1, an Endomorphin-2, a Leu-enkephalin, a Met-enkephalin, a Galanin, a Lofentanil or a Nociceptin.
61. The modified Clostridial toxin according to Claim 57, wherein the non-Clostridial toxin binding domain is selected from the group consisting of an antibody against the lactoseries carbohydrate epitopes found on the surface of dorsal root ganglion neurons (e.g. monoclonal antibodies 1B2 and LA4), an antibody against any of the receptors for the binding domains given above or an antibody against the surface expressed antigen Thyl (e.g. monoclonal antibody MRC OX7).

FIG. 1.

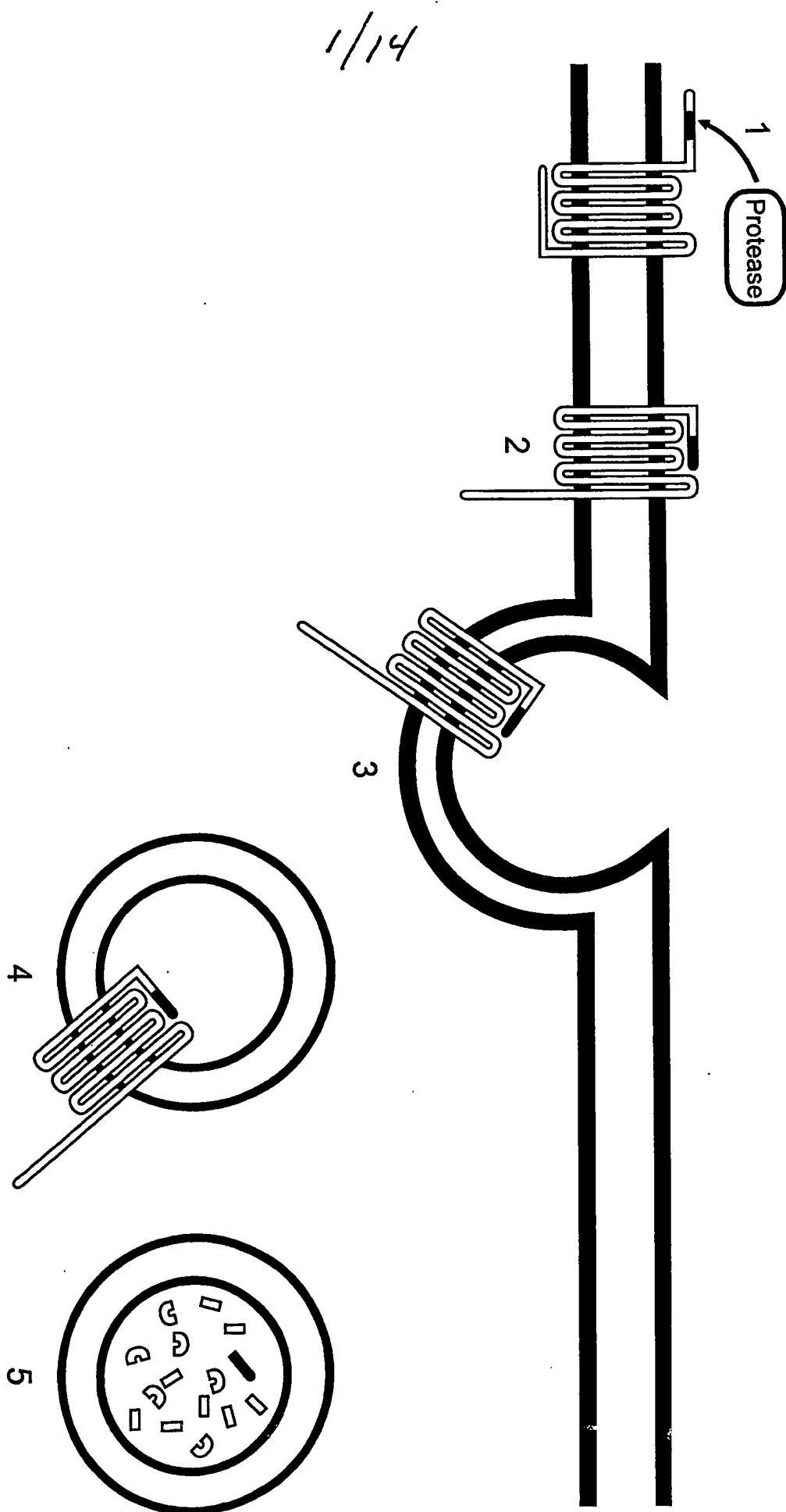
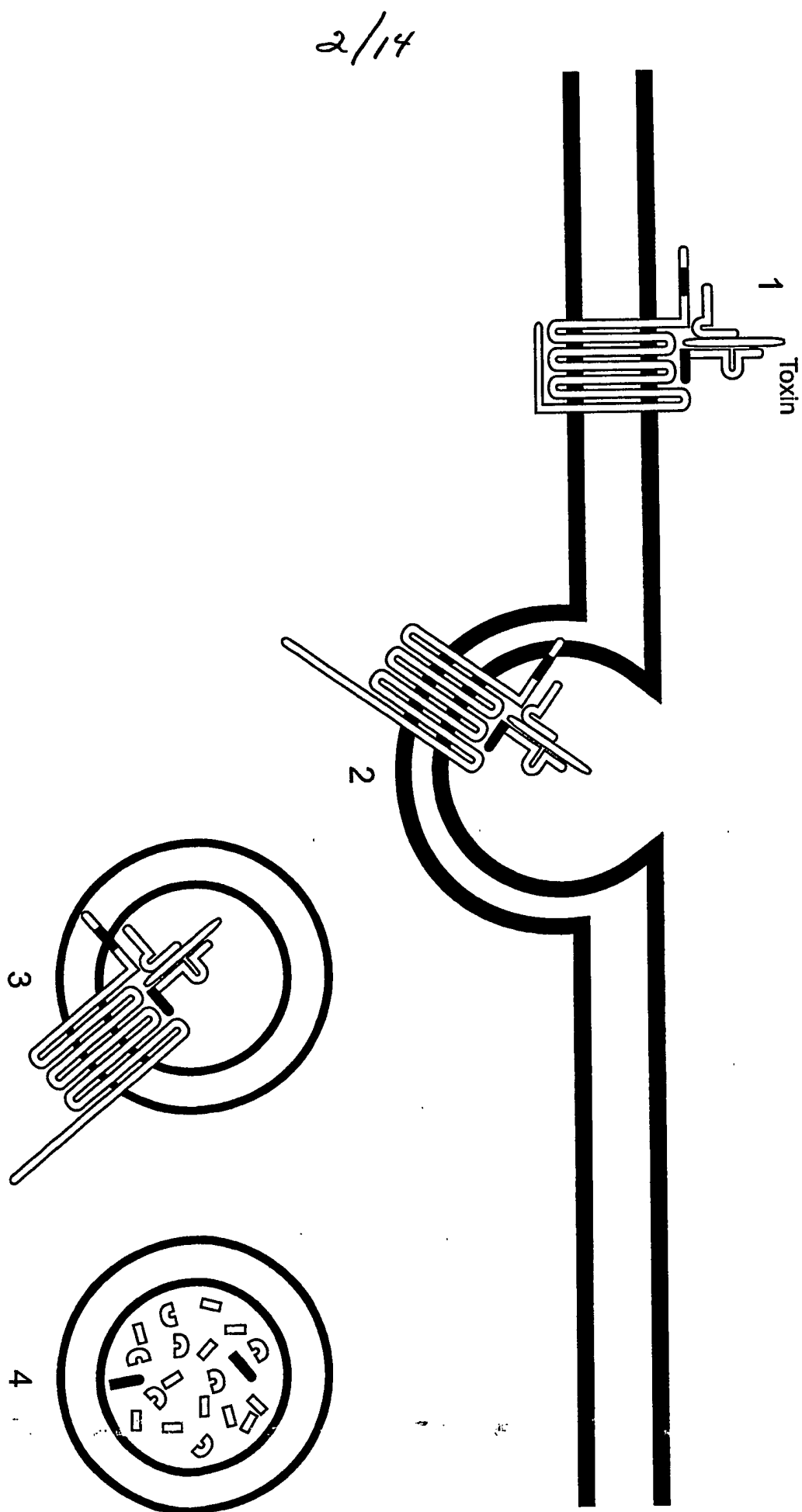
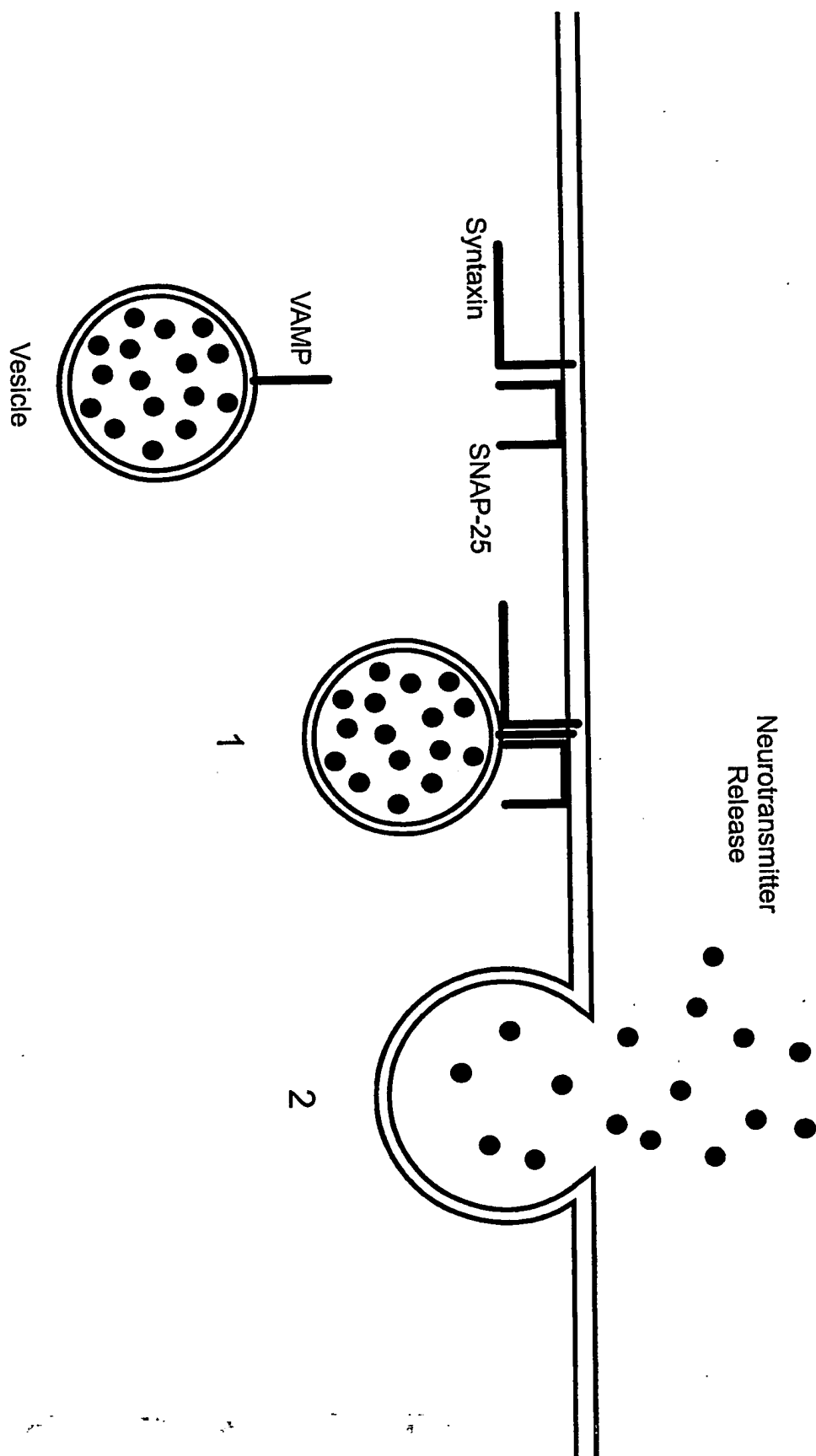


FIG. 2.



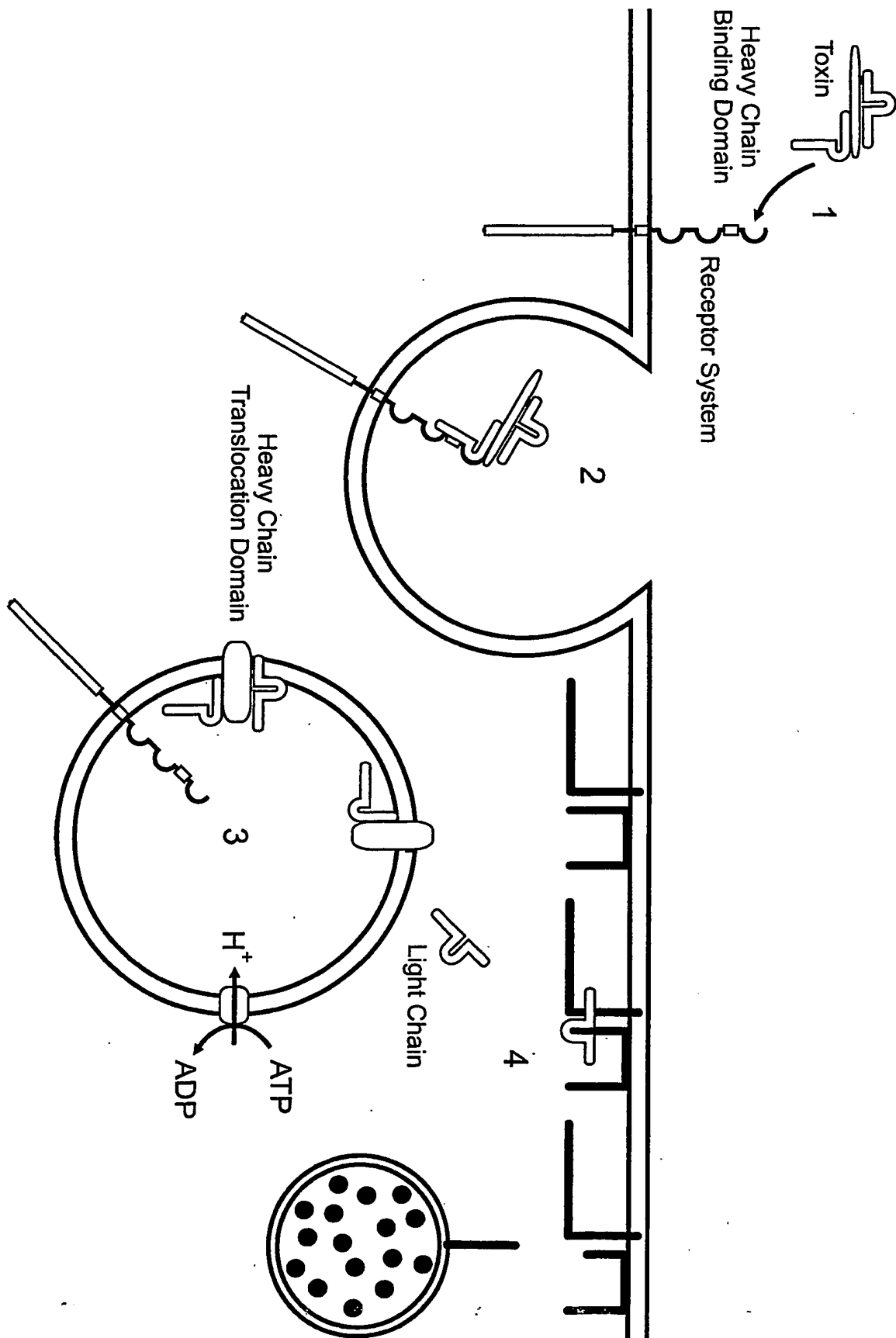
3/14

FIG. 3a.



4/14

FIG. 3b.





5/14

FIG. 4A.

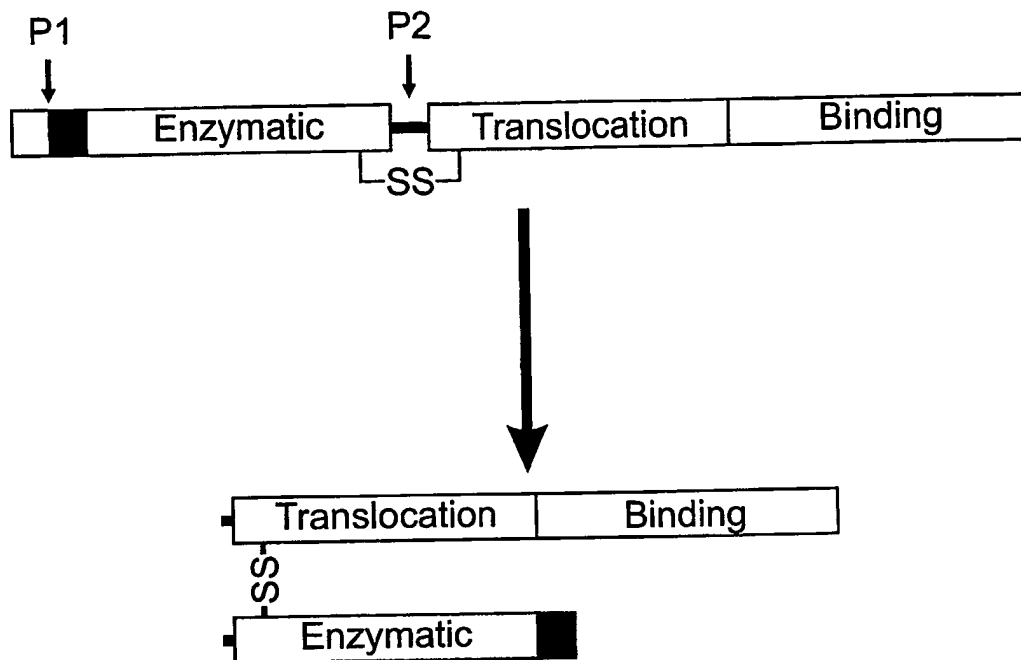
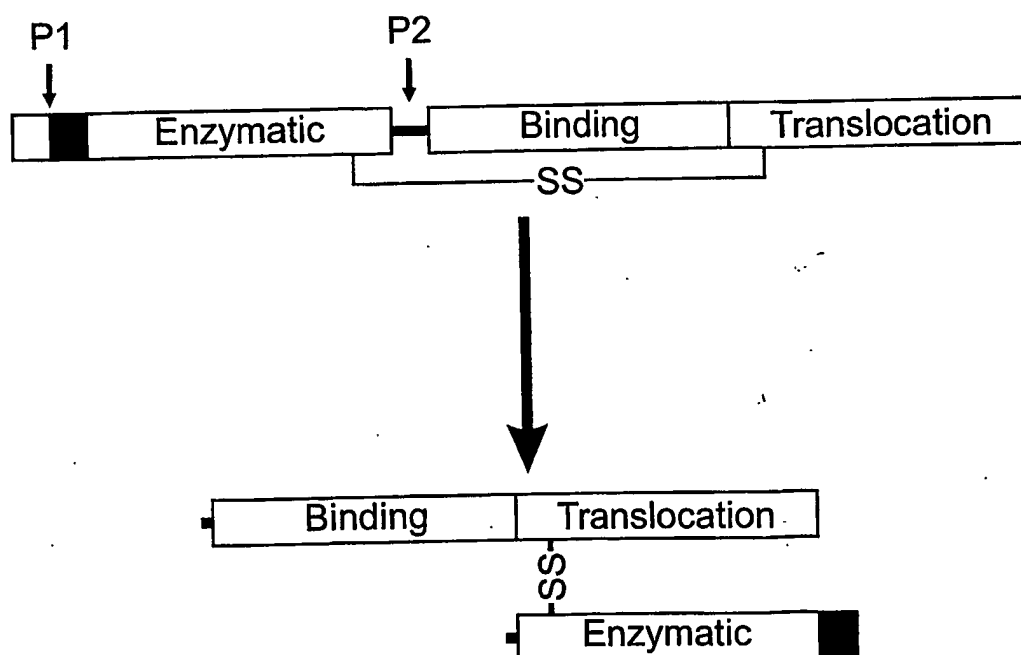


FIG. 4B.



16/14

FIG. 4C.

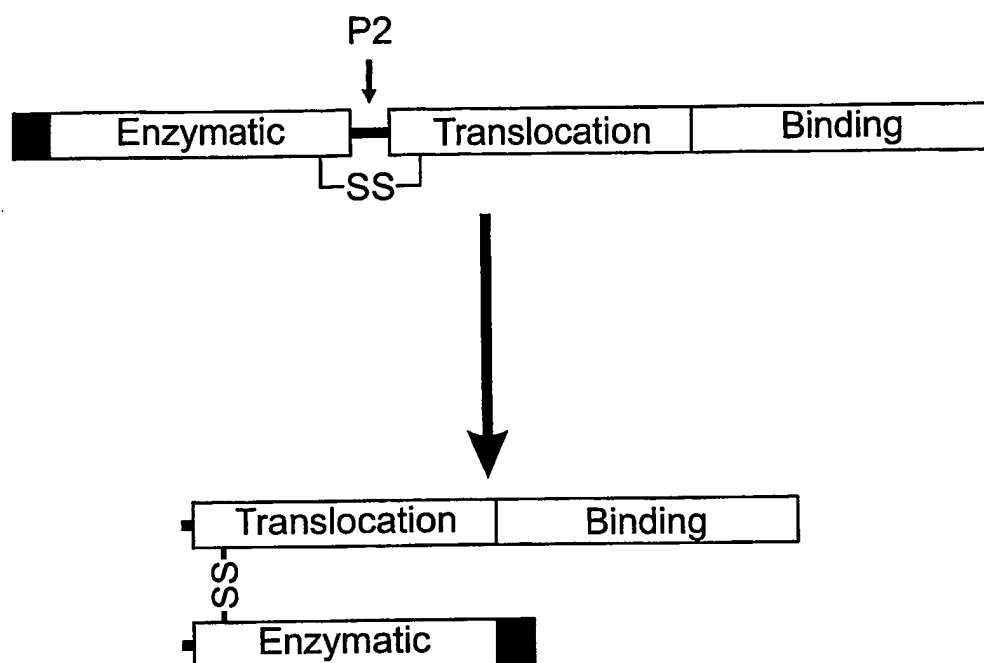
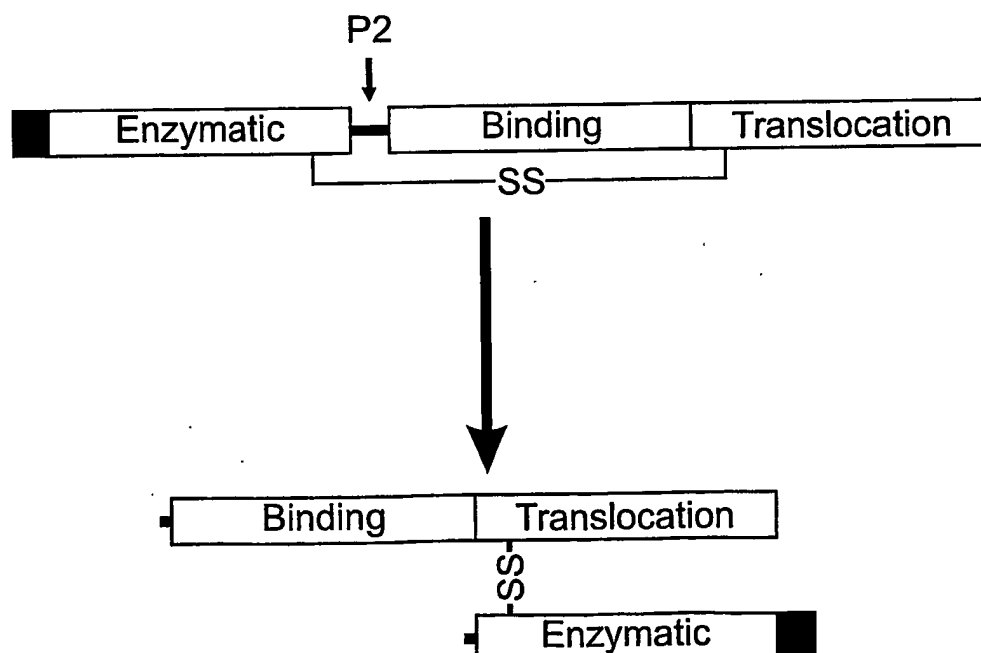


FIG. 4D.



7/14

FIG. 5A.

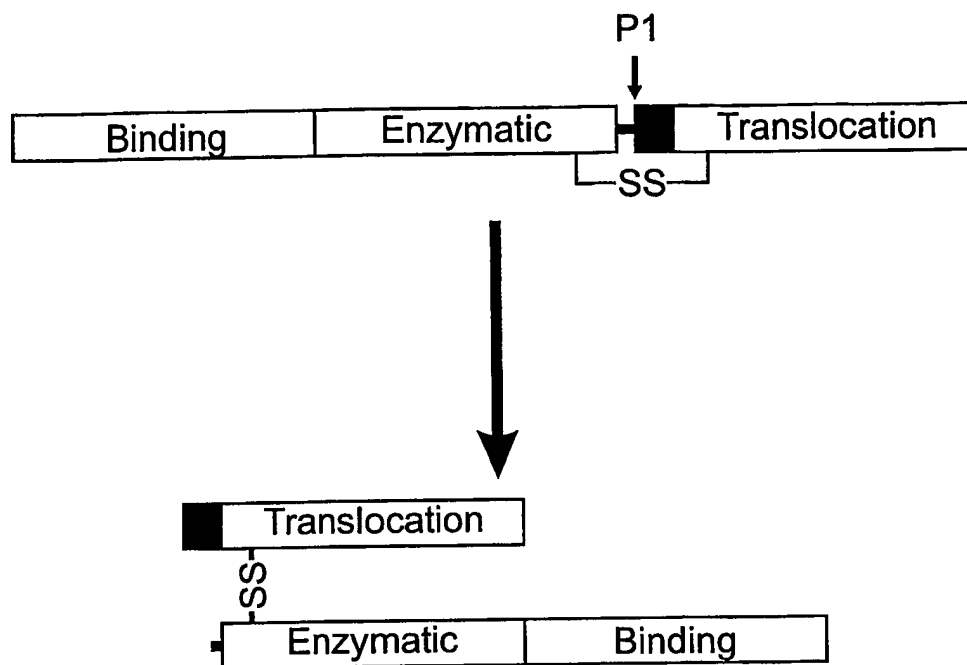
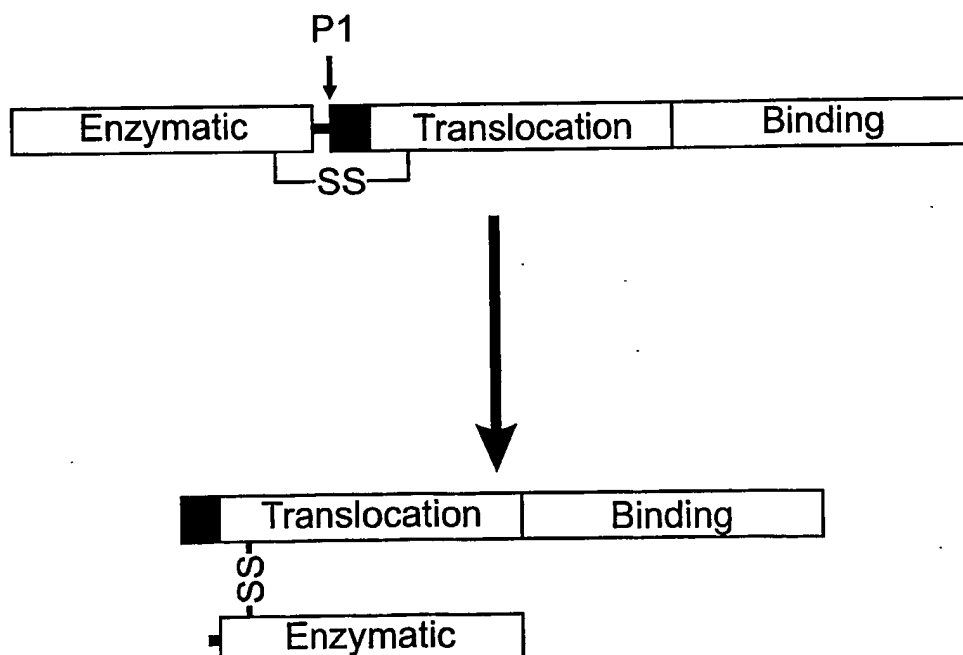
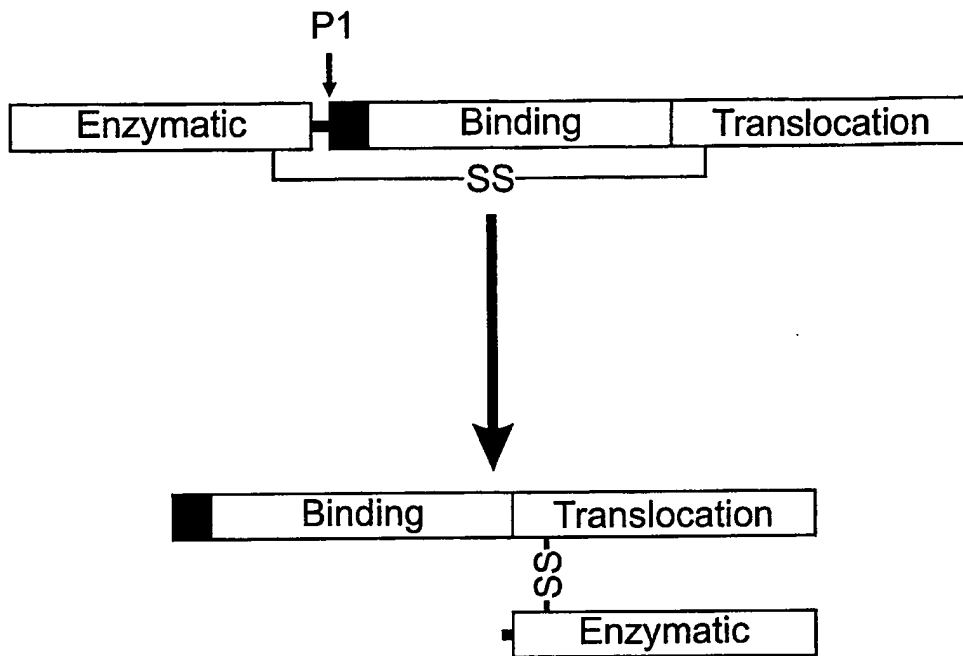


FIG. 5B.



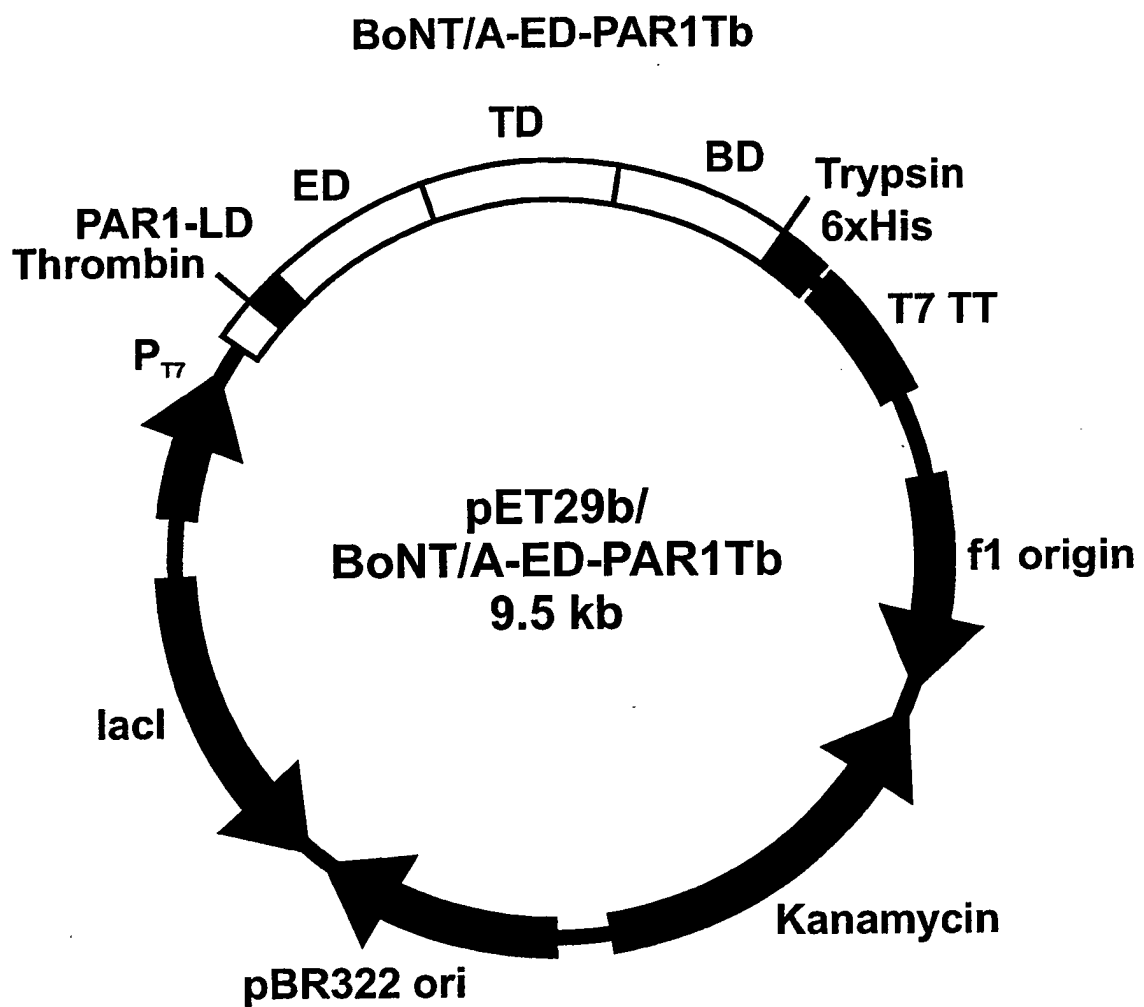
8/14

FIG. 6.



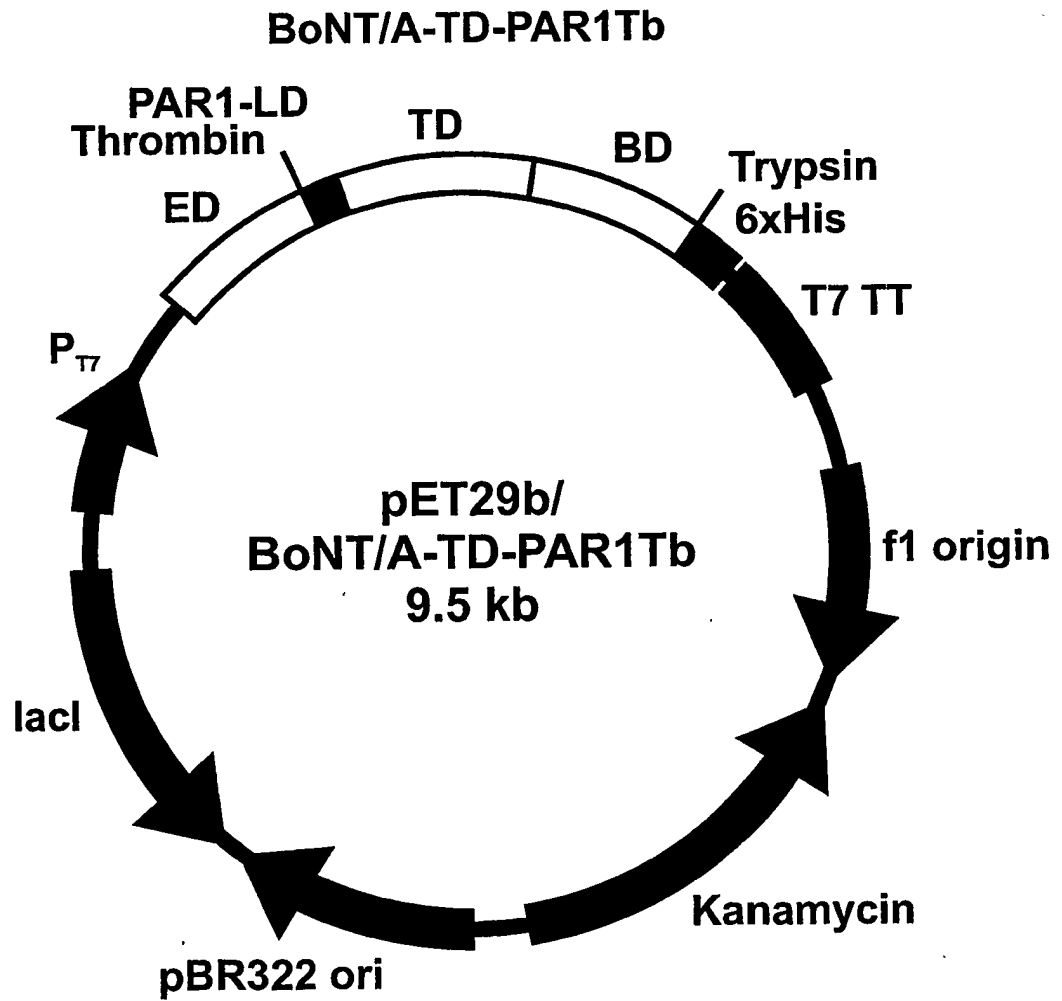
9/14

FIG. 7.



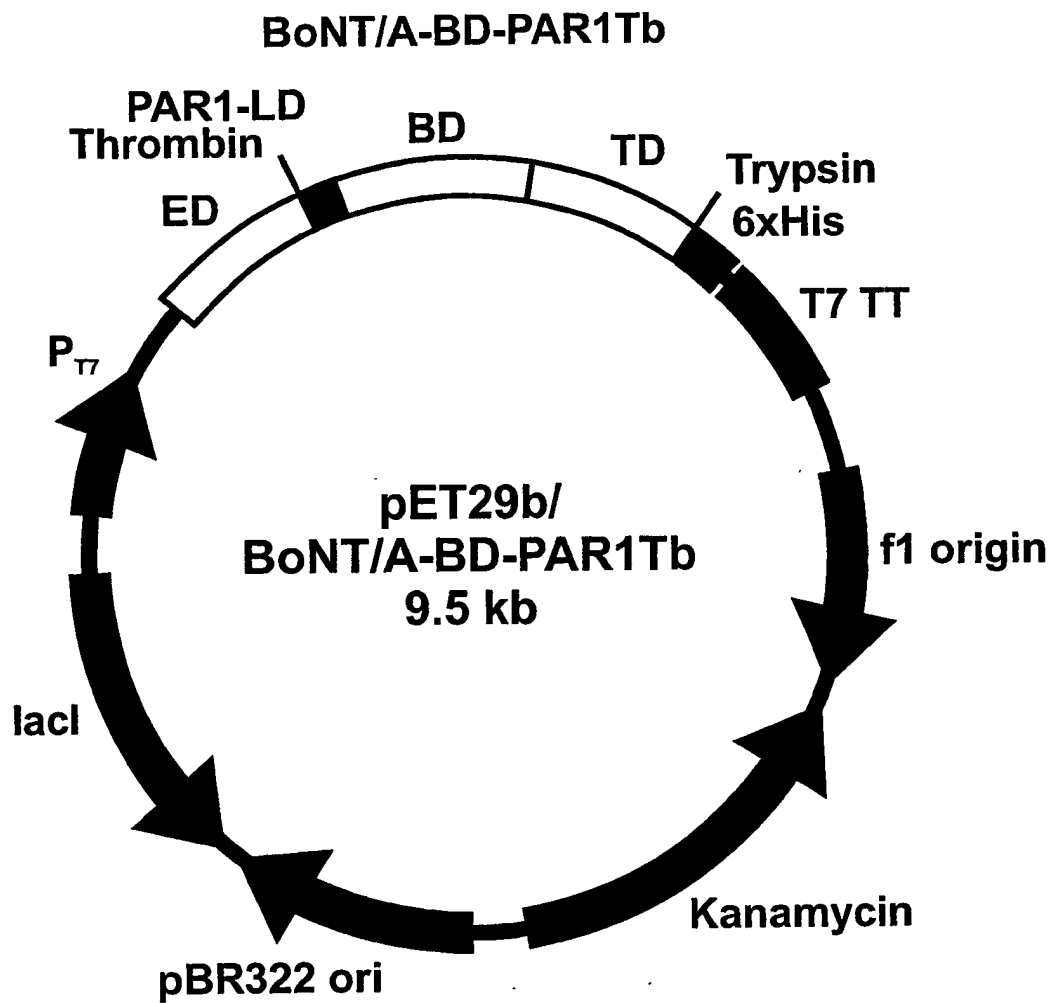
10/14

FIG. 8.



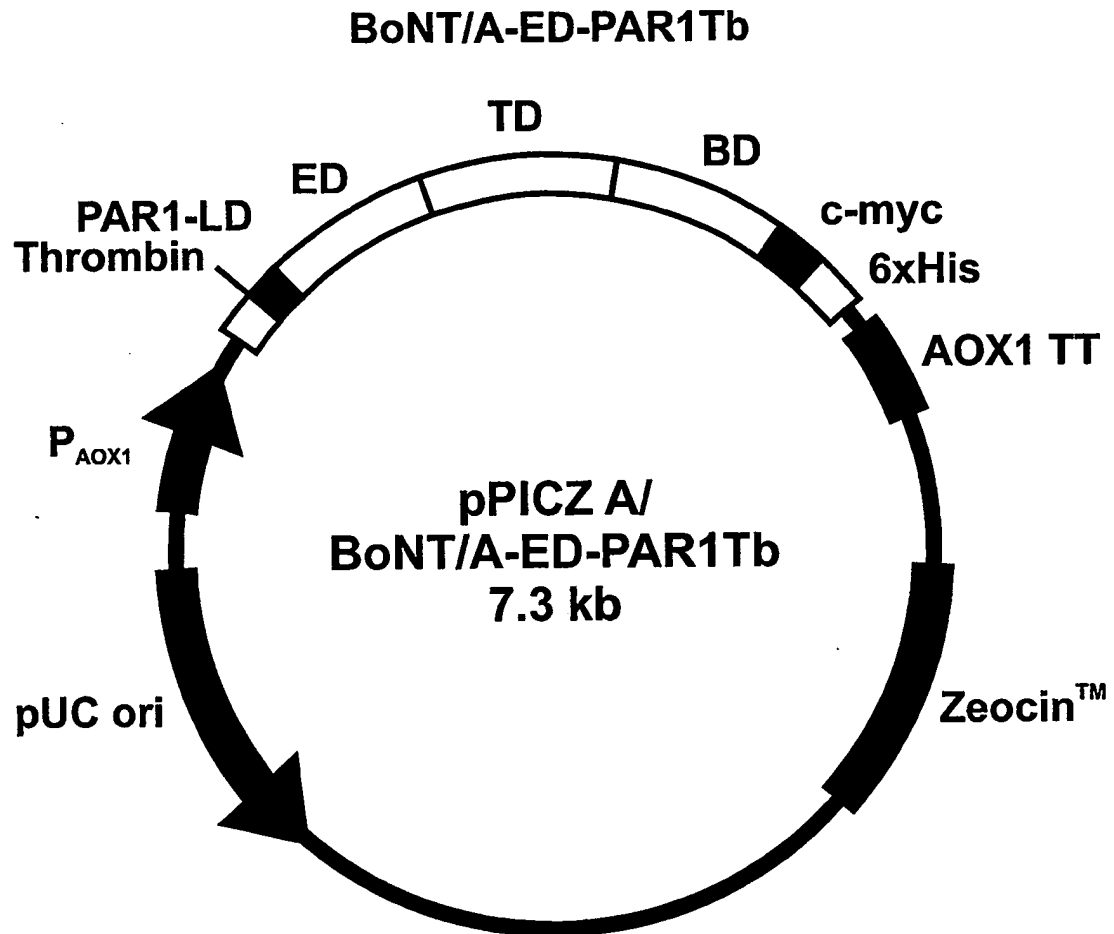
11/14

FIG. 9.



12/14

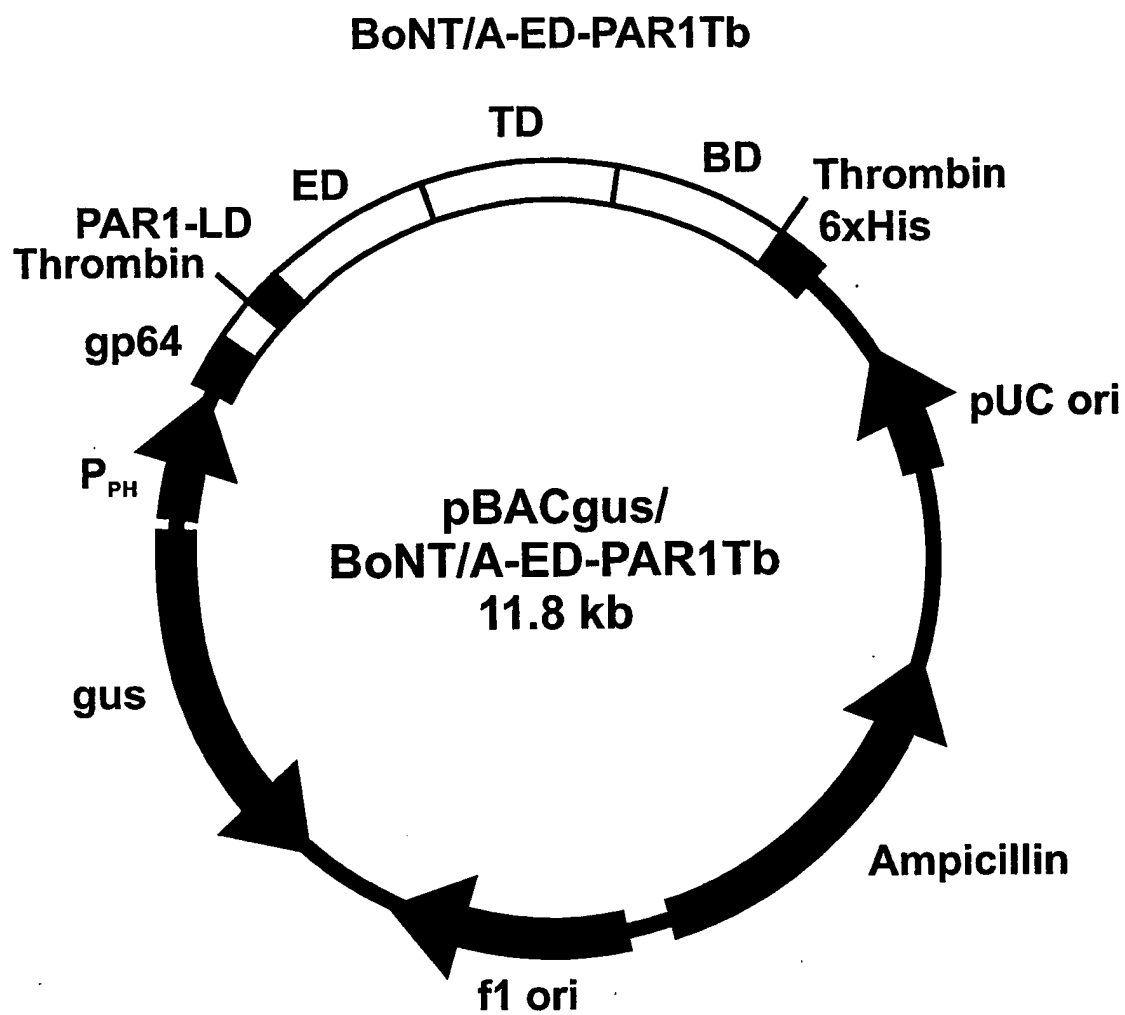
FIG. 10.





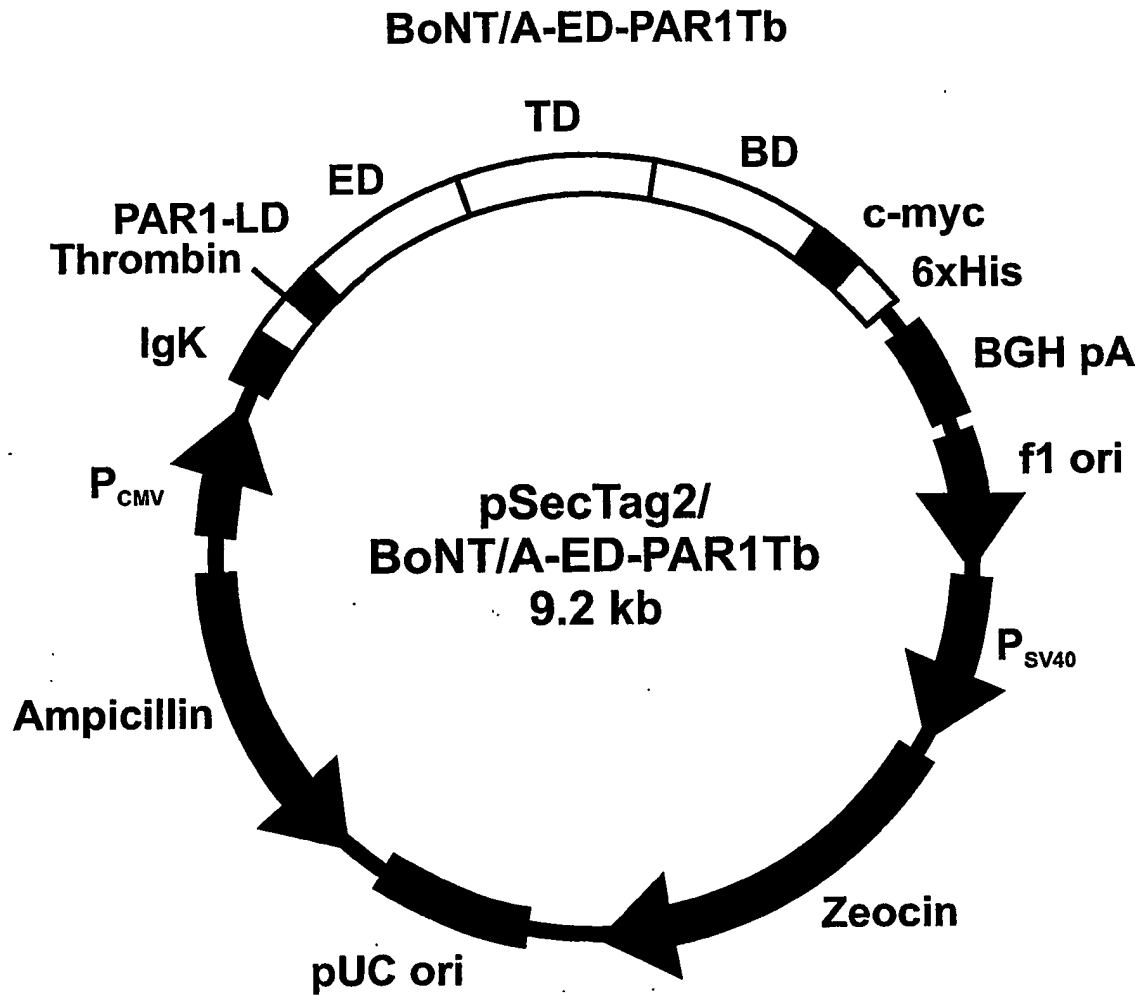
13/14

FIG. 11.



14/14

FIG. 12.



Li *et al.*, Degradable Clostridial Toxins

## SEQUENCE LISTING

<110> Li, Shengwen  
 Steward, Lance E.  
 Fernandez-Salas, Ester  
 Gilmore, Marcella  
 Francis, Joe  
 Aoki, Kei Roger

<120> Degradable Clostridial Toxins

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<221> DOMAIN  
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 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
 35 40 45  
 Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu  
 50 55 60  
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr  
 65 70 75 80  
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu  
 85 90 95  
 Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val  
 100 105 110  
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 115 120 125  
 Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr  
 130 135 140  
 Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile  
 145 150 155 160  
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Li *et al.*, Degradable Clostridial Toxins

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			245					250						255		
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	450					455					460					
Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	
465				470					475					480		
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			485					490						495		
Asp	Leu	Ile	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro		
			500				505					510				
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His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	
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Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	
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Li *et al.*, Degradable Clostridial Toxins

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	850		855		860	
Thr Phe Thr Glu	Tyr Ile Lys Asn Ile Ile Asn Thr	Ser Ile Leu Asn				
865		870		875		880
Leu Arg Tyr Glu	Ser Asn His Leu Ile Asp Leu Ser	Arg Tyr Ala Ser				
	885		890		895	
Lys Ile Asn Ile	Gly Ser Lys Val Asn Phe Asp Pro	Ile Asp Lys Asn				
	900		905		910	
Gln Ile Gln Leu	Phe Asn Leu Glu Ser Ser Lys Ile	Glu Val Ile Leu				
	915		920		925	
Lys Asn Ala Ile	Val Tyr Asn Ser Met Tyr Glu Asn Phe	Ser Thr Ser				
	930		935		940	
Phe Trp Ile Arg	Ile Pro Lys Tyr Phe Asn Ser Ile	Ser Leu Asn Asn				
945		950		955		960
Glu Tyr Thr Ile	Ile Asn Cys Met Glu Asn Asn Ser	Gly Trp Lys Val				
	965		970		975	
Ser Leu Asn Tyr	Gly Glu Ile Ile Trp Thr Leu Gln Asp	Thr Gln Glu				
	980		985		990	
Ile Lys Gln Arg	Val Val Phe Lys Tyr Ser Gln Met	Ile Asn Ile Ser				
	995		1000		1005	
Asp Tyr Ile Asn	Arg Trp Ile Phe Val Thr Ile Thr	Asn Asn Arg Leu				
	1010		1015		1020	
Asn Asn Ser Lys	Ile Tyr Ile Asn Gly Arg Leu Ile Asp	Gln Lys Pro				
1025		1030		1035		1040
Ile Ser Asn Leu	Gly Asn Ile His Ala Ser Asn Asn	Ile Met Phe Lys				
	1045		1050		1055	
Leu Asp Gly Cys	Arg Asp Thr His Arg Tyr Ile Trp	Ile Lys Tyr Phe				
	1060		1065		1070	
Asn Leu Phe Asp	Lys Glu Leu Asn Glu Lys Glu Ile	Lys Asp Leu Tyr				
	1075		1080		1085	
Asp Asn Gln Ser	Asn Ser Gly Ile Leu Lys Asp Phe	Trp Gly Asp Tyr				

Li *et al.*, Degradable Clostridial Toxins

1090	1095	1100
Leu Gln Tyr Asp Lys	Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn	
1105	1110	1115
Lys Tyr Val Asp Val	Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu	1120
	1125	1130
Lys Gly Pro Arg Gly	Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser	1135
	1140	1145
Ser Leu Tyr Arg Gly	Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly	1150
	1155	1160
Asn Lys Asp Asn Ile	Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val	1165
	1170	1175
Val Val Lys Asn Lys	Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala	1180
1185	1190	1195
Gly Val Glu Lys Ile	Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn	1200
	1205	1210
Leu Ser Gln Val Val	Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr	1215
	1220	1225
Asn Lys Cys Lys Met	Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly	1230
	1235	1240
Phe Ile Gly Phe His	Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser	1245
	1250	1255
Asn Trp Tyr Asn Arg	Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys	1260
1265	1270	1275
Ser Trp Glu Phe Ile	Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu	1280
	1285	1290
		1295

&lt;210&gt; 2

&lt;211&gt; 1291

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum Serotype B

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(441)

&lt;223&gt; Light chain comprising the enzymatic domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (442)...(847)

&lt;223&gt; Amino-terminal half of heavy chain comprising the translocation domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (848)...(1291)

&lt;223&gt; Carboxyl-terminal half of heavy chain comprising the binding domain.

&lt;400&gt; 2

Met Pro Val Thr Ile	Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn
1	5 10 15
Asn Asn Ile Ile Met	Met Glu Pro Pro Phe Ala Arg Gly Thr Gly Arg
	20 25 30
Tyr Tyr Lys Ala Phe	Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu
	35 40 45
Arg Tyr Thr Phe Gly	Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly
	50 55 60
Ile Phe Asn Arg Asp	Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn
65	70 75 80
Thr Asn Asp Lys Lys	Asn Ile Phe Leu Gln Thr Met Ile Lys Leu Phe

## Li et al., Degradable Clostridial Toxins

				85				90				95			
Asn	Arg	Ile	Lys	Ser	Lys	Pro	Leu	Gly	Glu	Lys	Leu	Leu	Glu	Met	Ile
			100					105					110		
Ile	Asn	Gly	Ile	Pro	Tyr	Leu	Gly	Asp	Arg	Arg	Val	Pro	Leu	Glu	Glu
		115					120					125			
Phe	Asn	Thr	Asn	Ile	Ala	Ser	Val	Thr	Val	Asn	Lys	Leu	Ile	Ser	Asn
		130					135				140				
Pro	Gly	Glu	Val	Glu	Arg	Lys	Lys	Gly	Ile	Phe	Ala	Asn	Leu	Ile	Ile
145					150					155					160
Phe	Gly	Pro	Gly	Pro	Val	Leu	Asn	Glu	Asn	Glu	Thr	Ile	Asp	Ile	Gly
				165						170					175
Ile	Gln	Asn	His	Phe	Ala	Ser	Arg	Glu	Gly	Phe	Gly	Gly	Ile	Met	Gln
			180					185					190		
Met	Lys	Phe	Cys	Pro	Glu	Tyr	Val	Ser	Val	Phe	Asn	Asn	Val	Gln	Glu
		195					200					205			
Asn	Lys	Gly	Ala	Ser	Ile	Phe	Asn	Arg	Arg	Gly	Tyr	Phe	Ser	Asp	Pro
		210					215				220				
Ala	Leu	Ile	Leu	Met	His	Glu	Leu	Ile	His	Val	Leu	His	Gly	Leu	Tyr
225					230					235					240
Gly	Ile	Lys	Val	Asp	Asp	Leu	Pro	Ile	Val	Pro	Asn	Glu	Lys	Lys	Phe
				245						250					255
Phe	Met	Gln	Ser	Thr	Asp	Ala	Ile	Gln	Ala	Glu	Glu	Leu	Tyr	Thr	Phe
			260					265					270		
Gly	Gly	Gln	Asp	Pro	Ser	Ile	Ile	Thr	Pro	Ser	Thr	Asp	Lys	Ser	Ile
		275					280					285			
Tyr	Asp	Lys	Val	Leu	Gln	Asn	Phe	Arg	Gly	Ile	Val	Asp	Arg	Leu	Asn
	290					295					300				
Lys	Val	Leu	Val	Cys	Ile	Ser	Asp	Pro	Asn	Ile	Asn	Ile	Asn	Ile	Tyr
305					310					315					320
Lys	Asn	Lys	Phe	Lys	Asp	Lys	Tyr	Lys	Phe	Val	Glu	Asp	Ser	Glu	Gly
			325							330					335
Lys	Tyr	Ser	Ile	Asp	Val	Glu	Ser	Phe	Asp	Lys	Leu	Tyr	Lys	Ser	Leu
			340					345					350		
Met	Phe	Gly	Phe	Thr	Glu	Thr	Asn	Ile	Ala	Glu	Asn	Tyr	Lys	Ile	Lys
		355					360					365			
Thr	Arg	Ala	Ser	Tyr	Phe	Ser	Asp	Ser	Leu	Pro	Pro	Val	Lys	Ile	Lys
	370					375						380			
Asn	Leu	Leu	Asp	Asn	Glu	Ile	Tyr	Thr	Ile	Glu	Glu	Gly	Phe	Asn	Ile
385					390					395					400
Ser	Asp	Lys	Asp	Met	Glu	Lys	Glu	Tyr	Arg	Gly	Gln	Asn	Lys	Ala	Ile
			405						410						415
Asn	Lys	Gln	Ala	Tyr	Glu	Glu	Ile	Ser	Lys	Glu	His	Leu	Ala	Val	Tyr
			420						425				430		
Lys	Ile	Gln	Met	Cys	Lys	Ser	Val	Lys	Ala	Pro	Gly	Ile	Cys	Ile	Asp
		435					440					445			
Val	Asp	Asn	Glu	Asp	Leu	Phe	Phe	Ile	Ala	Asp	Lys	Asn	Ser	Phe	Ser
	450					455					460				
Asp	Asp	Leu	Ser	Lys	Asn	Glu	Arg	Ile	Glu	Tyr	Asn	Thr	Gln	Ser	Asn
465					470					475					480
Tyr	Ile	Glu	Asn	Asp	Phe	Pro	Ile	Asn	Glu	Leu	Ile	Leu	Asp	Thr	Asp
			485						490						495
Leu	Ile	Ser	Lys	Ile	Glu	Leu	Pro	Ser	Glu	Asn	Thr	Glu	Ser	Leu	Thr
			500						505				510		
Asp	Phe	Asn	Val	Asp	Val	Pro	Val	Tyr	Glu	Lys	Gln	Pro	Ala	Ile	Lys
		515					520					525			
Lys	Ile	Phe	Thr	Asp	Glu	Asn	Thr	Ile	Phe	Gln	Tyr	Leu	Tyr	Ser	Gln
	530					535					540				
Thr	Phe	Pro	Leu	Asp	Ile	Arg	Asp	Ile	Ser	Leu	Thr	Ser	Ser	Phe	Asp
545					550					555					560

Li *et al.*, Degradable Clostridial Toxins

Asp	Ala	Leu	Leu	Phe	Ser	Asn	Lys	Val	Tyr	Ser	Phe	Phe	Ser	Met	Asp
				565					570					575	
Tyr	Ile	Lys	Thr	Ala	Asn	Lys	Val	Val	Glu	Ala	Gly	Leu	Phe	Ala	Gly
			580					585					590		
Trp	Val	Lys	Gln	Ile	Val	Asn	Asp	Phe	Val	Ile	Glu	Ala	Asn	Lys	Ser
		595					600					605			
Asn	Thr	Met	Asp	Lys	Ile	Ala	Asp	Ile	Ser	Leu	Ile	Val	Pro	Tyr	Ile
	610					615					620				
Gly	Leu	Ala	Leu	Asn	Val	Gly	Asn	Glu	Thr	Ala	Lys	Gly	Asn	Phe	Glu
625				630						635					640
Asn	Ala	Phe	Glu	Ile	Ala	Gly	Ala	Ser	Ile	Leu	Leu	Glu	Phe	Ile	Pro
			645					650						655	
Glu	Leu	Leu	Ile	Pro	Val	Val	Gly	Ala	Phe	Leu	Leu	Glu	Ser	Tyr	Ile
		660					665					670			
Asp	Asn	Lys	Asn	Lys	Ile	Ile	Lys	Thr	Ile	Asp	Asn	Ala	Leu	Thr	Lys
		675					680					685			
Arg	Asn	Glu	Lys	Trp	Ser	Asp	Met	Tyr	Gly	Leu	Ile	Val	Ala	Gln	Trp
	690					695					700				
Leu	Ser	Thr	Val	Asn	Thr	Gln	Phe	Tyr	Thr	Ile	Lys	Glu	Gly	Met	Tyr
705				710						715					720
Lys	Ala	Leu	Asn	Tyr	Gln	Ala	Gln	Ala	Leu	Glu	Glu	Ile	Ile	Lys	Tyr
			725					730						735	
Arg	Tyr	Asn	Ile	Tyr	Ser	Glu	Lys	Glu	Lys	Ser	Asn	Ile	Asn	Ile	Asp
		740					745					750			
Phe	Asn	Asp	Ile	Asn	Ser	Lys	Leu	Asn	Glu	Gly	Ile	Asn	Gln	Ala	Ile
	755					760					765				
Asp	Asn	Ile	Asn	Asn	Phe	Ile	Asn	Gly	Cys	Ser	Val	Ser	Tyr	Leu	Met
	770				775					780					
Lys	Lys	Met	Ile	Pro	Leu	Ala	Val	Glu	Lys	Leu	Leu	Asp	Phe	Asp	Asn
785				790						795					800
Thr	Leu	Lys	Lys	Asn	Leu	Leu	Asn	Tyr	Ile	Asp	Glu	Asn	Lys	Leu	Tyr
			805					810						815	
Leu	Ile	Gly	Ser	Ala	Glu	Tyr	Glu	Lys	Ser	Lys	Val	Asn	Lys	Tyr	Leu
		820					825					830			
Lys	Thr	Ile	Met	Pro	Phe	Asp	Leu	Ser	Ile	Tyr	Thr	Asn	Asp	Thr	Ile
	835						840					845			
Leu	Ile	Glu	Met	Phe	Asn	Lys	Tyr	Asn	Ser	Glu	Ile	Leu	Asn	Asn	Ile
	850					855					860				
Ile	Leu	Asn	Leu	Arg	Tyr	Lys	Asp	Asn	Asn	Leu	Ile	Asp	Leu	Ser	Gly
865				870						875					880
Tyr	Gly	Ala	Lys	Val	Glu	Val	Tyr	Asp	Gly	Val	Glu	Leu	Asn	Asp	Lys
			885					890						895	
Asn	Gln	Phe	Lys	Leu	Thr	Ser	Ser	Ala	Asn	Ser	Lys	Ile	Arg	Val	Thr
		900						905					910		
Gln	Asn	Gln	Asn	Ile	Ile	Phe	Asn	Ser	Val	Phe	Leu	Asp	Phe	Ser	Val
		915					920					925			
Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Lys	Asn	Asp	Gly	Ile	Gln	Asn
	930					935					940				
Tyr	Ile	His	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Lys	Asn	Asn	Ser
945				950						955					960
Gly	Trp	Lys	Ile	Ser	Ile	Arg	Gly	Asn	Arg	Ile	Ile	Trp	Thr	Leu	Ile
			965					970						975	
Asp	Ile	Asn	Gly	Lys	Thr	Lys	Ser	Val	Phe	Phe	Glu	Tyr	Asn	Ile	Arg
		980					985					990			
Glu	Asp	Ile	Ser	Glu	Tyr	Ile	Asn	Arg	Trp	Phe	Phe	Val	Thr	Ile	Thr
	995						1000					1005			
Asn	Asn	Leu	Asn	Asn	Ala	Lys	Ile	Tyr	Ile	Asn	Gly	Lys	Leu	Glu	Ser
	1010					1015					1020				
Asn	Thr	Asp	Ile	Lys	Asp	Ile	Arg	Glu	Val	Ile	Ala	Asn	Gly	Glu	Ile



## Li et al., Degradable Clostridial Toxins

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1025          1030          1035          1040
Ile Phe Lys Leu Asp Gly Asp Ile Asp Arg Thr Gln Phe Ile Trp Met
          1045          1050          1055
Lys Tyr Phe Ser Ile Phe Asn Thr Glu Leu Ser Gln Ser Asn Ile Glu
          1060          1065          1070
Glu Arg Tyr Lys Ile Gln Ser Tyr Ser Glu Tyr Leu Lys Asp Phe Trp
          1075          1080          1085
Gly Asn Pro Leu Met Tyr Asn Lys Glu Tyr Tyr Met Phe Asn Ala Gly
          1090          1095          1100
Asn Lys Asn Ser Tyr Ile Lys Leu Lys Lys Asp Ser Pro Val Gly Glu
1105          1110          1115          1120
Ile Leu Thr Arg Ser Lys Tyr Asn Gln Asn Ser Lys Tyr Ile Asn Tyr
          1125          1130          1135
Arg Asp Leu Tyr Ile Gly Glu Lys Phe Ile Ile Arg Arg Lys Ser Asn
          1140          1145          1150
Ser Gln Ser Ile Asn Asp Asp Ile Val Arg Lys Glu Asp Tyr Ile Tyr
          1155          1160          1165
Leu Asp Phe Phe Asn Leu Asn Gln Glu Trp Arg Val Tyr Thr Tyr Lys
          1170          1175          1180
Tyr Phe Lys Lys Glu Glu Glu Lys Leu Phe Leu Ala Pro Ile Ser Asp
1185          1190          1195          1200
Ser Asp Glu Phe Tyr Asn Thr Ile Gln Ile Lys Glu Tyr Asp Glu Gln
          1205          1210          1215
Pro Thr Tyr Ser Cys Gln Leu Leu Phe Lys Lys Asp Glu Glu Ser Thr
          1220          1225          1230
Asp Glu Ile Gly Leu Ile Gly Ile His Arg Phe Tyr Glu Ser Gly Ile
          1235          1240          1245
Val Phe Glu Glu Tyr Lys Asp Tyr Phe Cys Ile Ser Lys Trp Tyr Leu
          1250          1255          1260
Lys Glu Val Lys Arg Lys Pro Tyr Asn Leu Lys Leu Gly Cys Asn Trp
1265          1270          1275          1280
Gln Phe Ile Pro Lys Asp Glu Gly Trp Thr Glu
          1285          1290

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&lt;210&gt; 3

&lt;211&gt; 1291

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum Serotype C1

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(449)

&lt;223&gt; Light chain comprising the enzymatic domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (450)...(855)

&lt;223&gt; Amino-terminal half of heavy chain comprising the translocation domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (856)...(1291)

&lt;223&gt; Carboxyl-terminal half of heavy chain comprising the binding domain.

&lt;400&gt; 3

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Met Pro Ile Thr Ile Asn Asn Phe Asn Tyr Ser Asp Pro Val Asp Asn
 1          5          10          15
Lys Asn Ile Leu Tyr Leu Asp Thr His Leu Asn Thr Leu Ala Asn Glu

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Li *et al.*, Degradable Clostridial Toxins[illegible]

## Li et al., Degradable Clostridial Toxins

Val Asp Gln Val Ile Leu Ser Lys Asn Thr Ser Glu His Gly Gln Leu  
 500 505 510  
 Asp Leu Leu Tyr Pro Ser Ile Asp Ser Glu Ser Glu Ile Leu Pro Gly  
 515 520 525  
 Glu Asn Gln Val Phe Tyr Asp Asn Arg Thr Gln Asn Val Asp Tyr Leu  
 530 535 540  
 Asn Ser Tyr Tyr Tyr Leu Glu Ser Gln Lys Leu Ser Asp Asn Val Glu  
 545 550 555 560  
 Asp Phe Thr Phe Thr Arg Ser Ile Glu Glu Ala Leu Asp Asn Ser Ala  
 565 570 575  
 Lys Val Tyr Thr Tyr Phe Pro Thr Leu Ala Asn Lys Val Asn Ala Gly  
 580 585 590  
 Val Gln Gly Gly Leu Phe Leu Met Trp Ala Asn Asp Val Val Glu Asp  
 595 600 605  
 Phe Thr Thr Asn Ile Leu Arg Lys Asp Thr Leu Asp Lys Ile Ser Asp  
 610 615 620  
 Val Ser Ala Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Ser Asn  
 625 630 635 640  
 Ser Val Arg Arg Gly Asn Phe Thr Glu Ala Phe Ala Val Thr Gly Val  
 645 650 655  
 Thr Ile Leu Leu Glu Ala Phe Pro Glu Phe Thr Ile Pro Ala Leu Gly  
 660 665 670  
 Ala Phe Val Ile Tyr Ser Lys Val Gln Glu Arg Asn Glu Ile Ile Lys  
 675 680 685  
 Thr Ile Asp Asn Cys Leu Glu Gln Arg Ile Lys Arg Trp Lys Asp Ser  
 690 695 700  
 Tyr Glu Trp Met Met Gly Thr Trp Leu Ser Arg Ile Ile Thr Gln Phe  
 705 710 715 720  
 Asn Asn Ile Ser Tyr Gln Met Tyr Asp Ser Leu Asn Tyr Gln Ala Gly  
 725 730 735  
 Ala Ile Lys Ala Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser  
 740 745 750  
 Asp Lys Glu Asn Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu  
 755 760 765  
 Asp Val Lys Ile Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg  
 770 775 780  
 Glu Cys Ser Val Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile  
 785 790 795 800  
 Asp Glu Leu Asn Glu Phe Asp Arg Asn Thr Lys Ala Lys Leu Ile Asn  
 805 810 815  
 Leu Ile Asp Ser His Asn Ile Ile Leu Val Gly Glu Val Asp Lys Leu  
 820 825 830  
 Lys Ala Lys Val Asn Asn Ser Phe Gln Asn Thr Ile Pro Phe Asn Ile  
 835 840 845  
 Phe Ser Tyr Thr Asn Asn Ser Leu Leu Lys Asp Ile Ile Asn Glu Tyr  
 850 855 860  
 Phe Asn Asn Ile Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Arg Lys  
 865 870 875 880  
 Asn Thr Leu Val Asp Thr Ser Gly Tyr Asn Ala Glu Val Ser Glu Glu  
 885 890 895  
 Gly Asp Val Gln Leu Asn Pro Ile Phe Pro Phe Asp Phe Lys Leu Gly  
 900 905 910  
 Ser Ser Gly Glu Asp Arg Gly Lys Val Ile Val Thr Gln Asn Glu Asn  
 915 920 925  
 Ile Val Tyr Asn Ser Met Tyr Glu Ser Phe Ser Ile Ser Phe Trp Ile  
 930 935 940  
 Arg Ile Asn Lys Trp Val Ser Asn Leu Pro Gly Tyr Thr Ile Ile Asp  
 945 950 955 960  
 Ser Val Lys Asn Asn Ser Gly Trp Ser Ile Gly Ile Ile Ser Asn Phe

Li *et al.*, Degradable Clostridial Toxins

				965					970					975	
Leu	Val	Phe	Thr	Leu	Lys	Gln	Asn	Glu	Asp	Ser	Glu	Gln	Ser	Ile	Asn
				980					985					990	
Phe	Ser	Tyr	Asp	Ile	Ser	Asn	Asn	Ala	Pro	Gly	Tyr	Asn	Lys	Trp	Phe
				995				1000						1005	
Phe	Val	Thr	Val	Thr	Asn	Asn	Met	Met	Gly	Asn	Met	Lys	Ile	Tyr	Ile
				1010				1015						1020	
Asn	Gly	Lys	Leu	Ile	Asp	Thr	Ile	Lys	Val	Lys	Glu	Leu	Thr	Gly	Ile
				1025				1030						1035	
Asn	Phe	Ser	Lys	Thr	Ile	Thr	Phe	Glu	Ile	Asn	Lys	Ile	Pro	Asp	Thr
				1045					1050					1055	
Gly	Leu	Ile	Thr	Ser	Asp	Ser	Asp	Asn	Ile	Asn	Met	Trp	Ile	Arg	Asp
				1060					1065					1070	
Phe	Tyr	Ile	Phe	Ala	Lys	Glu	Leu	Asp	Gly	Lys	Asp	Ile	Asn	Ile	Leu
				1075					1080					1085	
Phe	Asn	Ser	Leu	Gln	Tyr	Thr	Asn	Val	Val	Lys	Asp	Tyr	Trp	Gly	Asn
				1090					1095					1100	
Asp	Leu	Arg	Tyr	Asn	Lys	Glu	Tyr	Tyr	Met	Val	Asn	Ile	Asp	Tyr	Leu
				1105					1110					1115	
Asn	Arg	Tyr	Met	Tyr	Ala	Asn	Ser	Arg	Gln	Ile	Val	Phe	Asn	Thr	Arg
				1125					1130					1135	
Arg	Asn	Asn	Asn	Asp	Phe	Asn	Glu	Gly	Tyr	Lys	Ile	Ile	Ile	Lys	Arg
				1140					1145					1150	
Ile	Arg	Gly	Asn	Thr	Asn	Asp	Thr	Arg	Val	Arg	Gly	Gly	Asp	Ile	Leu
				1155					1160					1165	
Tyr	Phe	Asp	Met	Thr	Ile	Asn	Asn	Lys	Ala	Tyr	Asn	Leu	Phe	Met	Lys
				1170					1175					1180	
Asn	Glu	Thr	Met	Tyr	Ala	Asp	Asn	His	Ser	Thr	Glu	Asp	Ile	Tyr	Ala
				1185					1190					1195	
Ile	Gly	Leu	Arg	Glu	Gln	Thr	Lys	Asp	Ile	Asn	Asp	Asn	Ile	Ile	Phe
				1205					1210					1215	
Gln	Ile	Gln	Pro	Met	Asn	Asn	Thr	Tyr	Tyr	Tyr	Ala	Ser	Gln	Ile	Phe
				1220					1225					1230	
Lys	Ser	Asn	Phe	Asn	Gly	Glu	Asn	Ile	Ser	Gly	Ile	Cys	Ser	Ile	Gly
				1235					1240					1245	
Thr	Tyr	Arg	Phe	Arg	Leu	Gly	Gly	Asp	Trp	Tyr	Arg	His	Asn	Tyr	Leu
				1250					1255					1260	
Val	Pro	Thr	Val	Lys	Gln	Gly	Asn	Tyr	Ala	Ser	Leu	Leu	Glu	Ser	Thr
				1265					1270					1275	
Ser	Thr	His	Trp	Gly	Phe	Val	Pro	Val	Ser	Glu					1280
				1285					1290						

&lt;210&gt; 4

&lt;211&gt; 1276

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum Serotype D

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(442)

&lt;223&gt; Light chain comprising the enzymatic domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (443)...(851)

&lt;223&gt; Amino-terminal half of heavy chain comprising the translocation domain.

&lt;221&gt; DOMAIN

Li *et al.*, Degradable Clostridial Toxins

&lt;222&gt; (852)...(1276)

&lt;223&gt; Carboxyl-terminal half of heavy chain comprising the binding domain.

&lt;400&gt; 4

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Met Thr Trp Pro Val Lys Asp Phe Asn Tyr Ser Asp Pro Val Asn Asp
 1           5           10           15
Asn Asp Ile Leu Tyr Leu Arg Ile Pro Gln Asn Lys Leu Ile Thr Thr
      20           25           30
Pro Val Lys Ala Phe Met Ile Thr Gln Asn Ile Trp Val Ile Pro Glu
      35           40           45
Arg Phe Ser Ser Asp Thr Asn Pro Ser Leu Ser Lys Pro Pro Arg Pro
      50           55           60
Thr Ser Lys Tyr Gln Ser Tyr Tyr Asp Pro Ser Tyr Leu Ser Thr Asp
      65           70           75           80
Glu Gln Lys Asp Thr Phe Leu Lys Gly Ile Ile Lys Leu Phe Lys Arg
      85           90           95
Ile Asn Glu Arg Asp Ile Gly Lys Lys Leu Ile Asn Tyr Leu Val Val
      100          105          110
Gly Ser Pro Phe Met Gly Asp Ser Ser Thr Pro Glu Asp Thr Phe Asp
      115          120          125
Phe Thr Arg His Thr Thr Asn Ile Ala Val Glu Lys Phe Glu Asn Gly
      130          135          140
Ser Trp Lys Val Thr Asn Ile Ile Thr Pro Ser Val Leu Ile Phe Gly
      145          150          155          160
Pro Leu Pro Asn Ile Leu Asp Tyr Thr Ala Ser Leu Thr Leu Gln Gly
      165          170          175
Gln Gln Ser Asn Pro Ser Phe Glu Gly Phe Gly Thr Leu Ser Ile Leu
      180          185          190
Lys Val Ala Pro Glu Phe Leu Leu Thr Phe Ser Asp Val Thr Ser Asn
      195          200          205
Gln Ser Ser Ala Val Leu Gly Lys Ser Ile Phe Cys Met Asp Pro Val
      210          215          220
Ile Ala Leu Met His Glu Leu Thr His Ser Leu His Gln Leu Tyr Gly
      225          230          235          240
Ile Asn Ile Pro Ser Asp Lys Arg Ile Arg Pro Gln Val Ser Glu Gly
      245          250          255
Phe Phe Ser Gln Asp Gly Pro Asn Val Gln Phe Glu Glu Leu Tyr Thr
      260          265          270
Phe Gly Gly Leu Asp Val Glu Ile Ile Pro Gln Ile Glu Arg Ser Gln
      275          280          285
Leu Arg Glu Lys Ala Leu Gly His Tyr Lys Asp Ile Ala Lys Arg Leu
      290          295          300
Asn Asn Ile Asn Lys Thr Ile Pro Ser Ser Trp Ile Ser Asn Ile Asp
      305          310          315          320
Lys Tyr Lys Lys Ile Phe Ser Glu Lys Tyr Asn Phe Asp Lys Asp Asn
      325          330          335
Thr Gly Asn Phe Val Val Asn Ile Asp Lys Phe Asn Ser Leu Tyr Ser
      340          345          350
Asp Leu Thr Asn Val Met Ser Glu Val Val Tyr Ser Ser Gln Tyr Asn
      355          360          365
Val Lys Asn Arg Thr His Tyr Phe Ser Arg His Tyr Leu Pro Val Phe
      370          375          380
Ala Asn Ile Leu Asp Asp Asn Ile Tyr Thr Ile Arg Asp Gly Phe Asn
      385          390          395          400
Leu Thr Asn Lys Gly Phe Asn Ile Glu Asn Ser Gly Gln Asn Ile Glu
      405          410          415
Arg Asn Pro Ala Leu Gln Lys Leu Ser Ser Glu Ser Val Val Asp Leu

```

## Lé et al., Degradable Clostridial Toxins

			420					425					430				
Phe	Thr	Lys	Val	Cys	Leu	Arg	Leu	Thr	Lys	Asn	Ser	Arg	Asp	Asp	Ser		
			435										445				
Thr	Cys	Ile	Lys	Val	Lys	Asn	Asn	Arg	Leu	Pro	Tyr	Val	Ala	Asp	Lys		
			450										460				
Asp	Ser	Ile	Ser	Gln	Glu	Ile	Phe	Glu	Asn	Lys	Ile	Ile	Thr	Asp	Glu		
Thr	Asn	Val	Gln	Asn	Tyr	Ser	Asp	Lys	Phe	Ser	Leu	Asp	Glu	Ser	Ile		
Leu	Asp	Gly	Gln	Val	Pro	Ile	Asn	Pro	Glu	Ile	Val	Asp	Pro	Leu	Leu		
Pro	Asn	Val	Asn	Met	Glu	Pro	Leu	Asn	Leu	Pro	Gly	Glu	Glu	Ile	Val		
Phe	Tyr	Asp	Asp	Ile	Thr	Lys	Tyr	Val	Asp	Tyr	Leu	Asn	Ser	Tyr	Tyr		
Tyr	Leu	Glu	Ser	Gln	Lys	Leu	Ser	Asn	Asn	Val	Glu	Asn	Ile	Thr	Leu		
Thr	Thr	Ser	Val	Glu	Glu	Ala	Leu	Gly	Tyr	Ser	Asn	Lys	Ile	Tyr	Thr		
Phe	Leu	Pro	Ser	Leu	Ala	Glu	Lys	Val	Asn	Lys	Gly	Val	Gln	Ala	Gly		
Leu	Phe	Leu	Asn	Trp	Ala	Asn	Glu	Val	Val	Glu	Asp	Phe	Thr	Thr	Asn		
Ile	Met	Lys	Lys	Asp	Thr	Leu	Asp	Lys	Ile	Ser	Asp	Val	Ser	Val	Ile		
Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Ser	Ala	Leu	Arg		
Gly	Asn	Phe	Asn	Gln	Ala	Phe	Ala	Thr	Ala	Gly	Val	Ala	Phe	Leu	Leu		
Glu	Gly	Phe	Pro	Glu	Phe	Thr	Ile	Pro	Ala	Leu	Gly	Val	Phe	Thr	Phe		
Tyr	Ser	Ser	Ile	Gln	Glu	Arg	Glu	Lys	Ile	Ile	Lys	Thr	Ile	Glu	Asn		
Cys	Leu	Glu	Gln	Arg	Val	Lys	Arg	Trp	Lys	Asp	Ser	Tyr	Gln	Trp	Met		
Val	Ser	Asn	Trp	Leu	Ser	Arg	Ile	Thr	Thr	Gln	Phe	Asn	His	Ile	Asn		
Tyr	Gln	Met	Tyr	Asp	Ser	Leu	Ser	Tyr	Gln	Ala	Asp	Ala	Ile	Lys	Ala		
Lys	Ile	Asp	Leu	Glu	Tyr	Lys	Lys	Tyr	Ser	Gly	Ser	Asp	Lys	Glu	Asn		
Ile	Lys	Ser	Gln	Val	Glu	Asn	Leu	Lys	Asn	Ser	Leu	Asp	Val	Lys	Ile		
Ser	Glu	Ala	Met	Asn	Asn	Ile	Asn	Lys	Phe	Ile	Arg	Glu	Cys	Ser	Val		
Thr	Tyr	Leu	Phe	Lys	Asn	Met	Leu	Pro	Lys	Val	Ile	Asp	Glu	Leu	Asn		
Lys	Phe	Asp	Leu	Arg	Thr	Lys	Thr	Glu	Leu	Ile	Asn	Leu	Ile	Asp	Ser		
His	Asn	Ile	Ile	Leu	Val	Gly	Glu	Val	Asp	Arg	Leu	Lys	Ala	Lys	Val		
Asn	Glu	Ser	Phe	Glu	Asn	Thr	Met	Pro	Phe	Asn	Ile	Phe	Ser	Tyr	Thr		
Asn	Asn	Ser	Leu	Leu	Lys	Asp	Ile	Ile	Asn	Glu	Tyr	Phe	Asn	Ser	Ile		
Asn	Asp	Ser	Lys	Ile	Leu	Ser	Leu	Gln	Asn	Lys	Lys	Asn	Ala	Leu	Val		
Asp	Thr	Ser	Gly	Tyr	Asn	Ala	Glu	Val	Arg	Val	Gly	Asp	Asn	Val	Gln		



Li *et al.*, Degradable Clostridial Toxins

&lt;222&gt; (1)...(422)

&lt;223&gt; Light chain comprising the enzymatic domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (423)...(834)

&lt;223&gt; Amino-terminal half of heavy chain comprising the translocation domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (835)...(1252)

&lt;223&gt; Carboxyl-terminal half of heavy chain comprising the binding domain.

&lt;400&gt; 5

```

Met Pro Lys Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp Arg
 1           5           10           15
Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Glu Phe Tyr Lys Ser
 20           25           30
Phe Asn Ile Met Lys Asn Ile Trp Ile Ile Pro Glu Arg Asn Val Ile
 35           40           45
Gly Thr Thr Pro Gln Asp Phe His Pro Pro Thr Ser Leu Lys Asn Gly
 50           55           60
Asp Ser Ser Tyr Tyr Asp Pro Asn Tyr Leu Gln Ser Asp Glu Glu Lys
 65           70           75           80
Asp Arg Phe Leu Lys Ile Val Thr Lys Ile Phe Asn Arg Ile Asn Asn
 85           90           95
Asn Leu Ser Gly Ile Leu Leu Glu Leu Ser Lys Ala Asn Pro
 100          105          110
Tyr Leu Gly Asn Asp Asn Thr Pro Asp Asn Gln Phe His Ile Gly Asp
 115          120          125
Ala Ser Ala Val Glu Ile Lys Phe Ser Asn Gly Ser Gln Asp Ile Leu
 130          135          140
Leu Pro Asn Val Ile Ile Met Gly Ala Glu Pro Asp Leu Phe Glu Thr
 145          150          155          160
Asn Ser Ser Asn Ile Ser Leu Arg Asn Asn Tyr Met Pro Ser Asn His
 165          170          175
Gly Phe Gly Ser Ile Ala Ile Val Thr Phe Ser Pro Glu Tyr Ser Phe
 180          185          190
Arg Phe Asn Asp Asn Ser Met Asn Glu Phe Ile Gln Asp Pro Ala Leu
 195          200          205
Thr Leu Met His Glu Leu Ile His Ser Leu His Gly Leu Tyr Gly Ala
 210          215          220
Lys Gly Ile Thr Thr Lys Tyr Thr Ile Thr Gln Lys Gln Asn Pro Leu
 225          230          235          240
Ile Thr Asn Ile Arg Gly Thr Asn Ile Glu Glu Phe Leu Thr Phe Gly
 245          250          255
Gly Thr Asp Leu Asn Ile Ile Thr Ser Ala Gln Ser Asn Asp Ile Tyr
 260          265          270
Thr Asn Leu Leu Ala Asp Tyr Lys Lys Ile Ala Ser Lys Leu Ser Lys
 275          280          285
Val Gln Val Ser Asn Pro Leu Leu Asn Pro Tyr Lys Asp Val Phe Glu
 290          295          300
Ala Lys Tyr Gly Leu Asp Lys Asp Ala Ser Gly Ile Tyr Ser Val Asn
 305          310          315          320
Ile Asn Lys Phe Asn Asp Ile Phe Lys Lys Leu Tyr Ser Phe Thr Glu
 325          330          335
Phe Asp Leu Ala Thr Lys Phe Gln Val Lys Cys Arg Gln Thr Tyr Ile
 340          345          350

```



## Li et al., Degradable Clostridial Toxins

Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile  
 355 360 365  
 Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe  
 370 375 380  
 Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr  
 385 390 395 400  
 Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val  
 405 410 415  
 Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly  
 420 425 430  
 Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile  
 435 440 445  
 Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr  
 450 455 460  
 Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala  
 465 470 475 480  
 Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala  
 485 490 495  
 Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His  
 500 505 510  
 Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val  
 515 520 525  
 Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala  
 530 535 540  
 Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile  
 545 550 555 560  
 Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Ser Trp Ile  
 565 570 575  
 Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr  
 580 585 590  
 Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu  
 595 600 605  
 Ala Leu Asn Ile Gly Asn Glu Ala Gln Lys Gly Asn Phe Lys Asp Ala  
 610 615 620  
 Leu Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Glu Pro Glu Leu  
 625 630 635 640  
 Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser  
 645 650 655  
 Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys  
 660 665 670  
 Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn  
 675 680 685  
 Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met  
 690 695 700  
 Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Ile Lys Thr Ile Ile Glu  
 705 710 715 720  
 Ser Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Asn Glu Leu Thr Asn  
 725 730 735  
 Lys Tyr Asp Ile Lys Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser  
 740 745 750  
 Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser  
 755 760 765  
 Tyr Leu Met Lys Leu Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu  
 770 775 780  
 Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asn Tyr Ile Ile Gln His  
 785 790 795 800  
 Gly Ser Ile Leu Gly Glu Ser Gln Gln Glu Leu Asn Ser Met Val Thr  
 805 810 815  
 Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp

## Li et al., Degradable Clostridial Toxins

	820		825		830
Asp Lys Ile Leu Ile Ser Tyr Phe Asn Lys Phe Phe Lys Arg Ile Lys					
	835		840		845
Ser Ser Ser Val Leu Asn Met Arg Tyr Lys Asn Asp Lys Tyr Val Asp					
	850		855		860
Thr Ser Gly Tyr Asp Ser Asn Ile Asn Ile Asn Gly Asp Val Tyr Lys					
865		870		875	880
Tyr Pro Thr Asn Lys Asn Gln Phe Gly Ile Tyr Asn Asp Lys Leu Ser					
	885		890		895
Glu Val Asn Ile Ser Gln Asn Asp Tyr Ile Ile Tyr Asp Asn Lys Tyr					
	900		905		910
Lys Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Asn Tyr Asp Asn					
	915		920		925
Lys Ile Val Asn Val Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Arg					
	930		935		940
Asp Asn Asn Ser Gly Trp Lys Val Ser Leu Asn His Asn Glu Ile Ile					
945		950		955	960
Trp Thr Leu Gln Asp Asn Ala Gly Ile Asn Gln Lys Leu Ala Phe Asn					
	965		970		975
Tyr Gly Asn Ala Asn Gly Ile Ser Asp Tyr Ile Asn Lys Trp Ile Phe					
	980		985		990
Val Thr Ile Thr Asn Asp Arg Leu Gly Asp Ser Lys Leu Tyr Ile Asn					
	995		1000		1005
Gly Asn Leu Ile Asp Gln Lys Ser Ile Leu Asn Leu Gly Asn Ile His					
	1010		1015		1020
Val Ser Asp Asn Ile Leu Phe Lys Ile Val Asn Cys Ser Tyr Thr Arg					
1025		1030		1035	1040
Tyr Ile Gly Ile Arg Tyr Phe Asn Ile Phe Asp Lys Glu Leu Asp Glu					
	1045		1050		1055
Thr Glu Ile Gln Thr Leu Tyr Ser Asn Glu Pro Asn Thr Asn Ile Leu					
	1060		1065		1070
Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asp Lys Glu Tyr Tyr Leu					
	1075		1080		1085
Leu Asn Val Leu Lys Pro Asn Asn Phe Ile Asp Arg Arg Lys Asp Ser					
	1090		1095		1100
Thr Leu Ser Ile Asn Asn Ile Arg Ser Thr Ile Leu Leu Ala Asn Arg					
1105		1110		1115	1120
Leu Tyr Ser Gly Ile Lys Val Lys Ile Gln Arg Val Asn Asn Ser Ser					
	1125		1130		1135
Thr Asn Asp Asn Leu Val Arg Lys Asn Asp Gln Val Tyr Ile Asn Phe					
	1140		1145		1150
Val Ala Ser Lys Thr His Leu Phe Pro Leu Tyr Ala Asp Thr Ala Thr					
	1155		1160		1165
Thr Asn Lys Glu Lys Thr Ile Lys Ile Ser Ser Ser Gly Asn Arg Phe					
	1170		1175		1180
Asn Gln Val Val Val Met Asn Ser Val Gly Asn Asn Cys Thr Met Asn					
1185		1190		1195	1200
Phe Lys Asn Asn Asn Gly Asn Asn Ile Gly Leu Leu Gly Phe Lys Ala					
	1205		1210		1215
Asp Thr Val Val Ala Ser Thr Trp Tyr Tyr Thr His Met Arg Asp His					
	1220		1225		1230
Thr Asn Ser Asn Gly Cys Phe Trp Asn Phe Ile Ser Glu Glu His Gly					
	1235		1240		1245
Trp Gln Glu Lys					
1250					

&lt;210&gt; 6

&lt;211&gt; 1274

## Li et al., Degradable Clostridial Toxins

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum Serotype F

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(436)

&lt;223&gt; Light chain comprising the enzymatic domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (437)...(852)

&lt;223&gt; Amino-terminal half of heavy chain comprising the translocation domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (853)...(1274)

&lt;223&gt; Carboxyl-terminal half of heavy chain comprising the binding domain.

&lt;400&gt; 6

```

Met Pro Val Ala Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp
 1           5           10           15
Asp Thr Ile Leu Tyr Met Gln Ile Pro Tyr Glu Glu Lys Ser Lys Lys
      20           25           30
Tyr Tyr Lys Ala Phe Glu Ile Met Arg Asn Val Trp Ile Ile Pro Glu
      35           40           45
Arg Asn Thr Ile Gly Thr Asn Pro Ser Asp Phe Asp Pro Pro Ala Ser
      50           55           60
Leu Lys Asn Gly Ser Ser Ala Tyr Tyr Asp Pro Asn Tyr Leu Thr Thr
      65           70           75           80
Asp Ala Glu Lys Asp Arg Tyr Leu Lys Thr Thr Ile Lys Leu Phe Lys
      85           90           95
Arg Ile Asn Ser Asn Pro Ala Gly Lys Val Leu Leu Gln Glu Ile Ser
      100          105          110
Tyr Ala Lys Pro Tyr Leu Gly Asn Asp His Thr Pro Ile Asp Glu Phe
      115          120          125
Ser Pro Val Thr Arg Thr Thr Ser Val Asn Ile Lys Leu Ser Thr Asn
      130          135          140
Val Glu Ser Ser Met Leu Leu Asn Leu Leu Val Leu Gly Ala Gly Pro
      145          150          155          160
Asp Ile Phe Glu Ser Cys Cys Tyr Pro Val Arg Lys Leu Ile Asp Pro
      165          170          175
Asp Val Val Tyr Asp Pro Ser Asn Tyr Gly Phe Gly Ser Ile Asn Ile
      180          185          190
Val Thr Phe Ser Pro Glu Tyr Glu Tyr Thr Phe Asn Asp Ile Ser Gly
      195          200          205
Gly His Asn Ser Ser Thr Glu Ser Phe Ile Ala Asp Pro Ala Ile Ser
      210          215          220
Leu Ala His Glu Leu Ile His Ala Leu His Gly Leu Tyr Gly Ala Arg
      225          230          235          240
Gly Val Thr Tyr Glu Glu Thr Ile Glu Val Lys Gln Ala Pro Leu Met
      245          250          255
Ile Ala Glu Lys Pro Ile Arg Leu Glu Glu Phe Leu Thr Phe Gly Gly
      260          265          270
Gln Asp Leu Asn Ile Ile Thr Ser Ala Met Lys Glu Lys Ile Tyr Asn
      275          280          285
Asn Leu Leu Ala Asn Tyr Glu Lys Ile Ala Thr Arg Leu Ser Glu Val
      290          295          300
Asn Ser Ala Pro Pro Glu Tyr Asp Ile Asn Glu Tyr Lys Asp Tyr Phe
      305          310          315          320

```

Li *et al.*, Degradable Clostridial Toxins

Gln Trp Lys Tyr Gly Leu Asp Lys Asn Ala Asp Gly Ser Tyr Thr Val  
 325 330 335  
 Asn Glu Asn Lys Phe Asn Glu Ile Tyr Lys Lys Leu Tyr Ser Phe Thr  
 340 345 350  
 Glu Ser Asp Leu Ala Asn Lys Phe Lys Val Lys Cys Arg Asn Thr Tyr  
 355 360 365  
 Phe Ile Lys Tyr Glu Phe Leu Lys Val Pro Asn Leu Leu Asp Asp Asp  
 370 375 380  
 Ile Tyr Thr Val Ser Glu Gly Phe Asn Ile Gly Asn Leu Ala Val Asn  
 385 390 395 400  
 Asn Arg Gly Gln Ser Ile Lys Leu Asn Pro Lys Ile Ile Asp Ser Ile  
 405 410 415  
 Pro Asp Lys Gly Leu Val Glu Lys Ile Val Lys Phe Cys Lys Ser Val  
 420 425 430  
 Ile Pro Arg Lys Gly Thr Lys Ala Pro Pro Arg Leu Cys Ile Arg Val  
 435 440 445  
 Asn Asn Ser Glu Leu Phe Phe Val Ala Ser Glu Ser Ser Tyr Asn Glu  
 450 455 460  
 Asn Asp Ile Asn Thr Pro Lys Glu Ile Asp Asp Thr Thr Asn Leu Asn  
 465 470 475 480  
 Asn Asn Tyr Arg Asn Asn Leu Asp Glu Val Ile Leu Asp Tyr Asn Ser  
 485 490 495  
 Gln Thr Ile Pro Gln Ile Ser Asn Arg Thr Leu Asn Thr Leu Val Gln  
 500 505 510  
 Asp Asn Ser Tyr Val Pro Arg Tyr Asp Ser Asn Gly Thr Ser Glu Ile  
 515 520 525  
 Glu Glu Tyr Asp Val Val Asp Phe Asn Val Phe Phe Tyr Leu His Ala  
 530 535 540  
 Gln Lys Val Pro Glu Gly Glu Thr Asn Ile Ser Leu Thr Ser Ser Ile  
 545 550 555 560  
 Asp Thr Ala Leu Leu Glu Glu Ser Lys Asp Ile Phe Phe Ser Ser Glu  
 565 570 575  
 Phe Ile Asp Thr Ile Asn Lys Pro Val Asn Ala Ala Leu Phe Ile Asp  
 580 585 590  
 Trp Ile Ser Lys Val Ile Arg Asp Phe Thr Thr Glu Ala Thr Gln Lys  
 595 600 605  
 Ser Thr Val Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr Val  
 610 615 620  
 Gly Leu Ala Leu Asn Ile Ile Ile Glu Ala Glu Lys Gly Asn Phe Glu  
 625 630 635 640  
 Glu Ala Phe Glu Leu Leu Gly Val Gly Ile Leu Leu Glu Phe Val Pro  
 645 650 655  
 Glu Leu Thr Ile Pro Val Ile Leu Val Phe Thr Ile Lys Ser Tyr Ile  
 660 665 670  
 Asp Ser Tyr Glu Asn Lys Asn Lys Ala Ile Lys Ala Ile Asn Asn Ser  
 675 680 685  
 Leu Ile Glu Arg Glu Ala Lys Trp Lys Glu Ile Tyr Ser Trp Ile Val  
 690 695 700  
 Ser Asn Trp Leu Thr Arg Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu  
 705 710 715 720  
 Gln Met Tyr Gln Ala Leu Gln Asn Gln Val Asp Ala Ile Lys Thr Ala  
 725 730 735  
 Ile Glu Tyr Lys Tyr Asn Asn Tyr Thr Ser Asp Glu Lys Asn Arg Leu  
 740 745 750  
 Glu Ser Glu Tyr Asn Ile Asn Asn Ile Glu Glu Glu Leu Asn Lys Lys  
 755 760 765  
 Val Ser Leu Ala Met Lys Asn Ile Glu Arg Phe Met Thr Glu Ser Ser  
 770 775 780  
 Ile Ser Tyr Leu Met Lys Leu Ile Asn Glu Ala Lys Val Gly Lys Leu

## Li et al., Degradable Clostridial Toxins

785		790		795		800
Lys Lys Tyr Asp	Asn His Val Lys Ser	Asp Leu Leu Asn Tyr	Ile Leu			
	805	810	815			
Asp His Arg Ser	Ile Leu Gly Glu Gln Thr	Asn Glu Leu Ser	Asp Leu			
	820	825	830			
Val Thr Ser Thr	Leu Asn Ser Ser	Ile Pro Phe Glu Leu Ser	Ser Tyr			
	835	840	845			
Thr Asn Asp Lys	Ile Leu Ile Ile Tyr Phe Asn Arg	Leu Tyr Lys Lys				
	850	855	860			
Ile Lys Asp Ser	Ser Ile Leu Asp Met Arg Tyr	Glu Asn Asn Lys Phe				
865	870	875	880			
Ile Asp Ile Ser	Gly Tyr Gly Ser Asn Ile Ser	Ile Asn Gly Asn Val				
	885	890	895			
Tyr Ile Tyr Ser	Thr Asn Arg Asn Gln Phe Gly	Ile Tyr Asn Ser Arg				
	900	905	910			
Leu Ser Glu Val	Asn Ile Ala Gln Asn Asn Asp	Ile Ile Tyr Asn Ser				
	915	920	925			
Arg Tyr Gln Asn	Phe Ser Ile Ser Phe Trp Val Arg	Ile Pro Lys His				
	930	935	940			
Tyr Lys Pro Met	Asn His Asn Arg Glu Tyr Thr	Ile Ile Asn Cys Met				
945	950	955	960			
Gly Asn Asn Asn	Ser Gly Trp Lys Ile Ser Leu Arg	Thr Val Arg Asp				
	965	970	975			
Cys Glu Ile Ile	Trp Thr Leu Gln Asp Thr Ser Gly	Asn Lys Glu Asn				
	980	985	990			
Leu Ile Phe Arg	Tyr Glu Glu Leu Asn Arg Ile Ser	Asn Tyr Ile Asn				
	995	1000	1005			
Lys Trp Ile Phe	Val Thr Ile Thr Asn Asn Arg Leu Gly	Asn Ser Arg				
	1010	1015	1020			
Ile Tyr Ile Asn	Gly Asn Leu Ile Val Glu Lys Ser	Ile Ser Asn Leu				
1025	1030	1035	1040			
Gly Asp Ile His	Val Ser Asp Asn Ile Leu Phe Lys	Ile Val Gly Cys				
	1045	1050	1055			
Asp Asp Glu Thr	Tyr Val Gly Ile Arg Tyr Phe Lys	Val Phe Asn Thr				
	1060	1065	1070			
Glu Leu Asp Lys	Thr Glu Ile Glu Thr Leu Tyr Ser	Asn Glu Pro Asp				
	1075	1080	1085			
Pro Ser Ile Leu	Lys Asn Tyr Trp Gly Asn Tyr Leu Leu Tyr	Asn Lys				
	1090	1095	1100			
Lys Tyr Tyr Leu	Phe Asn Leu Leu Arg Lys Asp Lys Tyr	Ile Thr Leu				
1105	1110	1115	1120			
Asn Ser Gly Ile	Leu Asn Ile Asn Gln Gln Arg Gly	Val Thr Glu Gly				
	1125	1130	1135			
Ser Val Phe Leu	Asn Tyr Lys Leu Tyr Glu Gly	Val Glu Val Ile Ile				
	1140	1145	1150			
Arg Lys Asn Gly	Pro Ile Asp Ile Ser Asn Thr Asp	Asn Phe Val Arg				
	1155	1160	1165			
Lys Asn Asp Leu	Ala Tyr Ile Asn Val Val Asp Arg Gly	Val Glu Tyr				
	1170	1175	1180			
Arg Leu Tyr Ala	Asp Thr Lys Ser Glu Lys Glu Lys	Ile Ile Arg Thr				
1185	1190	1195	1200			
Ser Asn Leu Asn	Asp Ser Leu Gly Gln Ile Ile Val Met	Asp Ser Ile				
	1205	1210	1215			
Gly Asn Asn Cys	Thr Met Asn Phe Gln Asn Asn Asn	Gly Ser Asn Ile				
	1220	1225	1230			
Gly Leu Leu Gly	Phe His Ser Asn Asn Leu Val Ala Ser	Ser Trp Tyr				
	1235	1240	1245			
Tyr Asn Asn Ile	Arg Arg Asn Thr Ser Ser Asn Gly	Cys Phe Trp Ser				
	1250	1255	1260			

## Li et al., Degradable Clostridial Toxins

Ser Ile Ser Lys Glu Asn Gly Trp Lys Glu  
1265 1270

```
<210> 7
<211> 1297
<212> PRT
<213> Clostridium botulinum Serotype G
```

```
<220>  
<221> DOMAIN  
<222> (1)...(442)  
<223> Light chain comprising the enzymatic domain.
```

```
<221> DOMAIN
<222> (443)...(852)
<223> Amino-terminal half of heavy chain comprising the
translocation domain.
```

```
<221> DOMAIN
<222> (853)...(1297)
<223> Carboxyl-terminal half of heavy chain comprising
the binding domain.
```

<400>	7														
Met 1	Pro	Val	Asn	Ile 5	Lys	Asn	Phe	Asn	Tyr 10	Asn	Asp	Pro	Ile	Asn 15	Asn
Asp	Asp	Ile	Ile	Met	Met	Glu	Pro	Phe	Asn 25	Asp	Pro	Gly	Pro	Gly 30	Thr
Tyr	Tyr	Lys 35	Ala	Phe	Arg	Ile	Ile 40	Asp	Arg	Ile	Trp	Ile 45	Val	Pro	Glu
Arg	Phe 50	Thr	Tyr	Gly	Phe	Gln 55	Pro	Asp	Gln	Phe	Asn 60	Ala	Ser	Thr	Gly
Val 65	Phe	Ser	Lys	Asp	Val 70	Tyr	Glu	Tyr	Tyr	Asp 75	Pro	Thr	Tyr	Leu 80	Lys
Thr	Asp	Ala	Glu	Lys 85	Asp	Lys	Phe	Leu	Lys 90	Thr	Met	Ile	Lys	Leu 95	Phe
Asn	Arg	Ile	Asn 100	Ser	Lys	Pro	Ser	Gly 105	Gln	Arg	Leu	Leu	Asp 110	Met	Ile
Val	Asp	Ala 115	Ile	Pro	Tyr	Leu	Gly 120	Asn	Ala	Ser	Thr	Pro 125	Pro	Asp	Lys
Phe 130	Ala	Ala	Asn	Val	Ala	Asn 135	Val	Ser	Ile	Asn	Lys 140	Lys	Ile	Ile	Gln
Pro 145	Gly	Ala	Glu	Asp	Gln 150	Ile	Lys	Gly	Leu	Met 155	Thr	Asn	Leu	Ile	Ile
Phe	Gly	Pro	Gly	Pro 165	Val	Leu	Ser	Asp	Asn 170	Phe	Thr	Asp	Ser	Met 175	Ile
Met	Asn	Gly	His 180	Ser	Pro	Ile	Ser	Glu 185	Gly	Phe	Gly	Ala	Arg 190	Met	Met
Ile	Arg	Phe 195	Cys	Pro	Ser	Cys	Leu 200	Asn	Val	Phe	Asn 205	Asn	Val	Gln	Glu
Asn	Lys 210	Asp	Thr	Ser	Ile	Phe 215	Ser	Arg	Arg	Ala	Tyr 220	Phe	Ala	Asp	Pro
Ala 225	Leu	Thr	Leu	Met	His 230	Glu	Leu	Ile	His	Val 235	Leu	His	Gly	Leu	Tyr
Gly	Ile	Lys	Ile	Ser 245	Asn	Leu	Pro	Ile	Thr 250	Pro	Asn	Thr	Lys	Glu 255	Phe
Phe	Met	Gln	His 260	Ser	Asp	Pro	Val	Gln 265	Ala	Glu	Glu	Leu	Tyr 270	Thr	Phe

## Li et al., Degradable Clostridial Toxins

Gly	Gly	His	Asp	Pro	Ser	Val	Ile	Ser	Pro	Ser	Thr	Asp	Met	Asn	Ile	275	280	285
Tyr	Asn	Lys	Ala	Leu	Gln	Asn	Phe	Gln	Asp	Ile	Ala	Asn	Arg	Leu	Asn	290	295	300
Ile	Val	Ser	Ser	Ala	Gln	Gly	Ser	Gly	Ile	Asp	Ile	Ser	Leu	Tyr	Lys	305	310	315
Gln	Ile	Tyr	Lys	Asn	Lys	Tyr	Asp	Phe	Val	Glu	Asp	Pro	Asn	Gly	Lys	325	330	335
Tyr	Ser	Val	Asp	Lys	Asp	Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Ala	Leu	Met	340	345	350
Phe	Gly	Phe	Thr	Glu	Thr	Asn	Leu	Ala	Gly	Glu	Tyr	Gly	Ile	Lys	Thr	355	360	365
Arg	Tyr	Ser	Tyr	Phe	Ser	Glu	Tyr	Leu	Pro	Pro	Ile	Lys	Thr	Glu	Lys	370	375	380
Leu	Leu	Asp	Asn	Thr	Ile	Tyr	Thr	Gln	Asn	Glu	Gly	Phe	Asn	Ile	Ala	385	390	395
Ser	Lys	Asn	Leu	Lys	Thr	Glu	Phe	Asn	Gly	Gln	Asn	Lys	Ala	Val	Asn	405	410	415
Lys	Glu	Ala	Tyr	Glu	Glu	Ile	Ser	Leu	Glu	His	Leu	Val	Ile	Tyr	Arg	420	425	430
Ile	Ala	Met	Cys	Lys	Pro	Val	Met	Tyr	Lys	Asn	Thr	Gly	Lys	Ser	Glu	435	440	445
Gln	Cys	Ile	Ile	Val	Asn	Asn	Glu	Asp	Leu	Phe	Phe	Ile	Ala	Asn	Lys	450	455	460
Asp	Ser	Phe	Ser	Lys	Asp	Leu	Ala	Lys	Ala	Glu	Thr	Ile	Ala	Tyr	Asn	465	470	475
Thr	Gln	Asn	Asn	Thr	Ile	Glu	Asn	Asn	Phe	Ser	Ile	Asp	Gln	Leu	Ile	485	490	495
Leu	Asp	Asn	Asp	Leu	Ser	Ser	Gly	Ile	Asp	Leu	Pro	Asn	Glu	Asn	Thr	500	505	510
Glu	Pro	Phe	Thr	Asn	Phe	Asp	Asp	Ile	Asp	Ile	Pro	Val	Tyr	Ile	Lys	515	520	525
Gln	Ser	Ala	Leu	Lys	Lys	Ile	Phe	Val	Asp	Gly	Asp	Ser	Leu	Phe	Glu	530	535	540
Tyr	Leu	His	Ala	Gln	Thr	Phe	Pro	Ser	Asn	Ile	Glu	Asn	Leu	Gln	Leu	545	550	555
Thr	Asn	Ser	Leu	Asn	Asp	Ala	Leu	Arg	Asn	Asn	Asn	Lys	Val	Tyr	Thr	565	570	575
Phe	Phe	Ser	Thr	Asn	Leu	Val	Glu	Lys	Ala	Asn	Thr	Val	Val	Gly	Ala	580	585	590
Ser	Leu	Phe	Val	Asn	Trp	Val	Lys	Gly	Val	Ile	Asp	Asp	Phe	Thr	Ser	595	600	605
Glu	Ser	Thr	Gln	Lys	Ser	Thr	Ile	Asp	Lys	Val	Ser	Asp	Val	Ser	Ile	610	615	620
Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Val	Gly	Asn	Glu	Thr	Ala	625	630	635
Lys	Glu	Asn	Phe	Lys	Asn	Ala	Phe	Glu	Ile	Gly	Gly	Ala	Ala	Ile	Leu	645	650	655
Met	Glu	Phe	Ile	Pro	Glu	Leu	Ile	Val	Pro	Ile	Val	Gly	Phe	Phe	Thr	660	665	670
Leu	Glu	Ser	Tyr	Val	Gly	Asn	Lys	Gly	His	Ile	Ile	Met	Thr	Ile	Ser	675	680	685
Asn	Ala	Leu	Lys	Lys	Arg	Asp	Gln	Lys	Trp	Thr	Asp	Met	Tyr	Gly	Leu	690	695	700
Ile	Val	Ser	Gln	Trp	Leu	Ser	Thr	Val	Asn	Thr	Gln	Phe	Tyr	Thr	Ile	705	710	715
Lys	Glu	Arg	Met	Tyr	Asn	Ala	Leu	Asn	Asn	Gln	Ser	Gln	Ala	Ile	Glu	725	730	735

Li *et al.*, Degradable Clostridial Toxins

Lys Ile Ile Glu Asp Gln Tyr Asn Arg Tyr Ser Glu Glu Asp Lys Met  
 740 745 750  
 Asn Ile Asn Ile Asp Phe Asn Asp Ile Asp Phe Lys Leu Asn Gln Ser  
 755 760 765  
 Ile Asn Leu Ala Ile Asn Asn Ile Asp Asp Phe Ile Asn Gln Cys Ser  
 770 775 780  
 Ile Ser Tyr Leu Met Asn Arg Met Ile Pro Leu Ala Val Lys Lys Leu  
 785 790 795 800  
 Lys Asp Phe Asp Asp Asn Leu Lys Arg Asp Leu Leu Glu Tyr Ile Asp  
 805 810 815  
 Thr Asn Glu Leu Tyr Leu Leu Asp Glu Val Asn Ile Leu Lys Ser Lys  
 820 825 830  
 Val Asn Arg His Leu Lys Asp Ser Ile Pro Phe Asp Leu Ser Leu Tyr  
 835 840 845  
 Thr Lys Asp Thr Ile Leu Ile Gln Val Phe Asn Asn Tyr Ile Ser Asn  
 850 855 860  
 Ile Ser Ser Asn Ala Ile Leu Ser Leu Ser Tyr Arg Gly Gly Arg Leu  
 865 870 875 880  
 Ile Asp Ser Ser Gly Tyr Gly Ala Thr Met Asn Val Gly Ser Asp Val  
 885 890 895  
 Ile Phe Asn Asp Ile Gly Asn Gly Gln Phe Lys Leu Asn Asn Ser Glu  
 900 905 910  
 Asn Ser Asn Ile Thr Ala His Gln Ser Lys Phe Val Val Tyr Asp Ser  
 915 920 925  
 Met Phe Asp Asn Phe Ser Ile Asn Phe Trp Val Arg Thr Pro Lys Tyr  
 930 935 940  
 Asn Asn Asn Asp Ile Gln Thr Tyr Leu Gln Asn Glu Tyr Thr Ile Ile  
 945 950 955 960  
 Ser Cys Ile Lys Asn Asp Ser Gly Trp Lys Val Ser Ile Lys Gly Asn  
 965 970 975  
 Arg Ile Ile Trp Thr Leu Ile Asp Val Asn Ala Lys Ser Lys Ser Ile  
 980 985 990  
 Phe Phe Glu Tyr Ser Ile Lys Asp Asn Ile Ser Asp Tyr Ile Asn Lys  
 995 1000 1005  
 Trp Phe Ser Ile Thr Ile Thr Asn Asp Arg Leu Gly Asn Ala Asn Ile  
 1010 1015 1020  
 Tyr Ile Asn Gly Ser Leu Lys Lys Ser Glu Lys Ile Leu Asn Leu Asp  
 1025 1030 1035 1040  
 Arg Ile Asn Ser Ser Asn Asp Ile Asp Phe Lys Leu Ile Asn Cys Thr  
 1045 1050 1055  
 Asp Thr Thr Lys Phe Val Trp Ile Lys Asp Phe Asn Ile Phe Gly Arg  
 1060 1065 1070  
 Glu Leu Asn Ala Thr Glu Val Ser Ser Leu Tyr Trp Ile Gln Ser Ser  
 1075 1080 1085  
 Thr Asn Thr Leu Lys Asp Phe Trp Gly Asn Pro Leu Arg Tyr Asp Thr  
 1090 1095 1100  
 Gln Tyr Tyr Leu Phe Asn Gln Gly Met Gln Asn Ile Tyr Ile Lys Tyr  
 1105 1110 1115 1120  
 Phe Ser Lys Ala Ser Met Gly Glu Thr Ala Pro Arg Thr Asn Phe Asn  
 1125 1130 1135  
 Asn Ala Ala Ile Asn Tyr Gln Asn Leu Tyr Leu Gly Leu Arg Phe Ile  
 1140 1145 1150  
 Ile Lys Lys Ala Ser Asn Ser Arg Asn Ile Asn Asn Asp Asn Ile Val  
 1155 1160 1165  
 Arg Glu Gly Asp Tyr Ile Tyr Leu Asn Ile Asp Asn Ile Ser Asp Glu  
 1170 1175 1180  
 Ser Tyr Arg Val Tyr Val Leu Val Asn Ser Lys Glu Ile Gln Thr Gln  
 1185 1190 1195 1200



Li *et al.*, Degradable Clostridial Toxins

Leu Phe Leu Ala Pro Ile Asn Asp Asp Pro Thr Phe Tyr Asp Val Leu  
                                   1205                                  1210                                  1215  
 Gln Ile Lys Lys Tyr Tyr Glu Lys Thr Thr Tyr Asn Cys Gln Ile Leu  
                                   1220                                  1225                                  1230  
 Cys Glu Lys Asp Thr Lys Thr Phe Gly Leu Phe Gly Ile Gly Lys Phe  
                                   1235                                  1240                                  1245  
 Val Lys Asp Tyr Gly Tyr Val Trp Asp Thr Tyr Asp Asn Tyr Phe Cys  
                                   1250                                  1255                                  1260  
 Ile Ser Gln Trp Tyr Leu Arg Arg Ile Ser Glu Asn Ile Asn Lys Leu  
 1265                                  1270                                  1275                                  1280  
 Arg Leu Gly Cys Asn Trp Gln Phe Ile Pro Val Asp Glu Gly Trp Thr  
                                   1285                                  1290                                  1295  
 Glu

&lt;210&gt; 8

&lt;211&gt; 1315

&lt;212&gt; PRT

&lt;213&gt; Clostridium tetani

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(441)

&lt;223&gt; Light chain comprising the enzymatic domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (442)...(870)

&lt;223&gt; Amino-terminal half of heavy chain comprising the translocation domain.

&lt;221&gt; DOMAIN

&lt;222&gt; (871)...(1315)

&lt;223&gt; Carboxyl-terminal half of heavy chain comprising the binding domain.

&lt;400&gt; 8

Met Pro Ile Thr Ile Asn Asn Phe Arg Tyr Ser Asp Pro Val Asn Asn  
   1                                  5                                  10                                  15  
 Asp Thr Ile Ile Met Met Glu Pro Pro Tyr Cys Lys Gly Leu Asp Ile  
                                   20                                  25                                  30  
 Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Val Pro Glu  
                                   35                                  40                                  45  
 Arg Tyr Glu Phe Gly Thr Lys Pro Glu Asp Phe Asn Pro Pro Ser Ser  
                                   50                                  55                                  60  
 Leu Ile Glu Gly Ala Ser Glu Tyr Tyr Asp Pro Asn Tyr Leu Arg Thr  
 65                                  70                                  75                                  80  
 Asp Ser Asp Lys Asp Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn  
                                   85                                  90                                  95  
 Arg Ile Lys Asn Asn Val Ala Gly Glu Ala Leu Leu Asp Lys Ile Ile  
                                   100                                  105                                  110  
 Asn Ala Ile Pro Tyr Leu Gly Asn Ser Tyr Ser Leu Leu Asp Lys Phe  
                                   115                                  120                                  125  
 Asp Thr Asn Ser Asn Ser Val Ser Phe Asn Leu Leu Glu Gln Asp Pro  
                                   130                                  135                                  140  
 Ser Gly Ala Thr Thr Lys Ser Ala Met Leu Thr Asn Leu Ile Ile Phe  
 145                                  150                                  155                                  160  
 Gly Pro Gly Pro Val Leu Asn Lys Asn Glu Val Arg Gly Ile Val Leu  
                                   165                                  170                                  175

## Li et al., Degradable Clostridial Toxins

Arg	Val	Asp	Asn	Lys	Asn	Tyr	Phe	Pro	Cys	Arg	Asp	Gly	Phe	Gly	Ser
			180					185					190		
Ile	Met	Gln	Met	Ala	Phe	Cys	Pro	Glu	Tyr	Val	Pro	Thr	Phe	Asp	Asn
		195					200					205			
Val	Ile	Glu	Asn	Ile	Thr	Ser	Leu	Thr	Ile	Gly	Lys	Ser	Lys	Tyr	Phe
		210				215					220				
Gln	Asp	Pro	Ala	Leu	Leu	Leu	Met	His	Glu	Leu	Ile	His	Val	Leu	His
225					230					235					240
Gly	Leu	Tyr	Gly	Met	Gln	Val	Ser	Ser	His	Glu	Ile	Ile	Pro	Ser	Lys
			245						250					255	
Gln	Glu	Ile	Tyr	Met	Gln	His	Thr	Tyr	Pro	Ile	Ser	Ala	Glu	Glu	Leu
			260					265					270		
Phe	Thr	Phe	Gly	Gly	Gln	Asp	Ala	Asn	Leu	Ile	Ser	Ile	Asp	Ile	Lys
		275					280					285			
Asn	Asp	Leu	Tyr	Glu	Lys	Thr	Leu	Asn	Asp	Tyr	Lys	Ala	Ile	Ala	Asn
		290				295					300				
Lys	Leu	Ser	Gln	Val	Thr	Ser	Cys	Asn	Asp	Pro	Asn	Ile	Asp	Ile	Asp
305					310					315					320
Ser	Tyr	Lys	Gln	Ile	Tyr	Gln	Gln	Lys	Tyr	Gln	Phe	Asp	Lys	Asp	Ser
			325					330					335		
Asn	Gly	Gln	Tyr	Ile	Val	Asn	Glu	Asp	Lys	Phe	Gln	Ile	Leu	Tyr	Asn
			340					345					350		
Ser	Ile	Met	Tyr	Gly	Phe	Thr	Glu	Ile	Glu	Leu	Gly	Lys	Lys	Phe	Asn
		355					360					365			
Ile	Lys	Thr	Arg	Leu	Ser	Tyr	Phe	Ser	Met	Asn	His	Asp	Pro	Val	Lys
		370				375					380				
Ile	Pro	Asn	Leu	Leu	Asp	Asp	Thr	Ile	Tyr	Asn	Asp	Thr	Glu	Gly	Phe
385					390					395					400
Asn	Ile	Glu	Ser	Lys	Asp	Leu	Lys	Ser	Glu	Tyr	Lys	Gly	Gln	Asn	Met
			405						410					415	
Arg	Val	Asn	Thr	Asn	Ala	Phe	Arg	Asn	Val	Asp	Gly	Ser	Gly	Leu	Val
			420					425					430		
Ser	Lys	Leu	Ile	Gly	Leu	Cys	Lys	Lys	Ile	Ile	Pro	Pro	Thr	Asn	Ile
		435					440					445			
Arg	Glu	Asn	Leu	Tyr	Asn	Arg	Thr	Ala	Ser	Leu	Thr	Asp	Leu	Gly	Gly
		450				455					460				
Glu	Leu	Cys	Ile	Lys	Ile	Lys	Asn	Glu	Asp	Leu	Thr	Phe	Ile	Ala	Glu
465					470				475						480
Lys	Asn	Ser	Phe	Ser	Glu	Glu	Pro	Phe	Gln	Asp	Glu	Ile	Val	Ser	Tyr
			485						490					495	
Asn	Thr	Lys	Asn	Lys	Pro	Leu	Asn	Phe	Asn	Tyr	Ser	Leu	Asp	Lys	Ile
			500					505					510		
Ile	Val	Asp	Tyr	Asn	Leu	Gln	Ser	Lys	Ile	Thr	Leu	Pro	Asn	Asp	Arg
		515					520					525			
Thr	Thr	Pro	Val	Thr	Lys	Gly	Ile	Pro	Tyr	Ala	Pro	Glu	Tyr	Lys	Ser
		530				535					540				
Asn	Ala	Ala	Ser	Thr	Ile	Glu	Ile	His	Asn	Ile	Asp	Asp	Asn	Thr	Ile
545					550					555					560
Tyr	Gln	Tyr	Leu	Tyr	Ala	Gln	Lys	Ser	Pro	Thr	Thr	Leu	Gln	Arg	Ile
			565						570					575	
Thr	Met	Thr	Asn	Ser	Val	Asp	Asp	Ala	Leu	Ile	Asn	Ser	Thr	Lys	Ile
			580					585					590		
Tyr	Ser	Tyr	Phe	Pro	Ser	Val	Ile	Ser	Lys	Val	Asn	Gln	Gly	Ala	Gln
		595					600					605			
Gly	Ile	Leu	Phe	Leu	Gln	Trp	Val	Arg	Asp	Ile	Ile	Asp	Asp	Phe	Thr
					615						620				
Asn	Glu	Ser	Ser	Gln	Lys	Thr	Thr	Ile	Asp	Lys	Ile	Ser	Asp	Val	Ser
625					630					635					640
Thr	Ile	Val	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Val	Lys	Gln	Gly

## Li et al., Degradable Clostridial Toxins

				645					650				655			
Tyr	Glu	Gly	Asn	Phe	Ile	Gly	Ala	Leu	Glu	Thr	Thr	Gly	Val	Val	Leu	
			660						665				670			
Leu	Leu	Glu	Tyr	Ile	Pro	Glu	Ile	Thr	Leu	Pro	Val	Ile	Ala	Ala	Leu	
		675					680					685				
Ser	Ile	Ala	Glu	Ser	Ser	Thr	Gln	Lys	Glu	Lys	Ile	Ile	Lys	Thr	Ile	
	690					695					700					
Asp	Asn	Phe	Leu	Glu	Lys	Arg	Tyr	Glu	Lys	Trp	Ile	Glu	Val	Tyr	Lys	
705					710					715					720	
Leu	Val	Lys	Ala	Lys	Trp	Leu	Gly	Thr	Val	Asn	Thr	Gln	Phe	Gln	Lys	
			725						730					735		
Arg	Ser	Tyr	Gln	Met	Tyr	Arg	Ser	Leu	Glu	Tyr	Gln	Val	Asp	Ala	Ile	
			740					745					750			
Lys	Lys	Ile	Asp	Tyr	Glu	Tyr	Lys	Ile	Tyr	Ser	Gly	Pro	Asp	Lys		
		755				760					765					
Glu	Gln	Ile	Ala	Asp	Glu	Ile	Asn	Asn	Leu	Lys	Asn	Lys	Leu	Glu	Glu	
	770					775					780					
Lys	Ala	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Ile	Phe	Met	Arg	Glu	Ser	
785					790					795					800	
Ser	Arg	Ser	Phe	Leu	Val	Asn	Gln	Met	Ile	Asn	Glu	Ala	Lys	Lys	Gln	
			805						810					815		
Leu	Leu	Glu	Phe	Asp	Thr	Gln	Ser	Lys	Asn	Ile	Leu	Met	Gln	Tyr	Ile	
			820					825					830			
Lys	Ala	Asn	Ser	Lys	Phe	Ile	Gly	Ile	Thr	Glu	Leu	Lys	Lys	Leu	Glu	
		835					840					845				
Ser	Lys	Ile	Asn	Lys	Val	Phe	Ser	Thr	Pro	Ile	Pro	Phe	Ser	Tyr	Ser	
	850					855					860					
Lys	Asn	Leu	Asp	Cys	Trp	Val	Asp	Asn	Glu	Glu	Asp	Ile	Asp	Val	Ile	
865					870					875					880	
Leu	Lys	Lys	Ser	Thr	Ile	Leu	Asn	Leu	Asp	Ile	Asn	Asn	Asp	Ile	Ile	
			885						890					895		
Ser	Asp	Ile	Ser	Gly	Phe	Asn	Ser	Ser	Val	Ile	Thr	Tyr	Pro	Asp	Ala	
			900					905					910			
Gln	Leu	Val	Pro	Gly	Ile	Asn	Gly	Lys	Ala	Ile	His	Leu	Val	Asn	Asn	
		915					920						925			
Glu	Ser	Ser	Glu	Val	Ile	Val	His	Lys	Ala	Met	Asp	Ile	Glu	Tyr	Asn	
	930					935					940					
Asp	Met	Phe	Asn	Asn	Phe	Thr	Val	Ser	Phe	Trp	Leu	Arg	Val	Pro	Lys	
945					950					955					960	
Val	Ser	Ala	Ser	His	Leu	Glu	Gln	Tyr	Gly	Thr	Asn	Glu	Tyr	Ser	Ile	
			965						970					975		
Ile	Ser	Ser	Met	Lys	Lys	His	Ser	Leu	Ser	Ile	Gly	Ser	Gly	Trp	Ser	
			980					985					990			
Val	Ser	Leu	Lys	Gly	Asn	Asn	Leu	Ile	Trp	Thr	Leu	Lys	Asp	Ser	Ala	
		995					1000						1005			
Gly	Glu	Val	Arg	Gln	Ile	Thr	Phe	Arg	Asp	Leu	Pro	Asp	Lys	Phe	Asn	
	1010					1015					1020					
Ala	Tyr	Leu	Ala	Asn	Lys	Trp	Val	Phe	Ile	Thr	Ile	Thr	Asn	Asp	Arg	
1025					1030					1035					1040	
Leu	Ser	Ser	Ala	Asn	Leu	Tyr	Ile	Asn	Gly	Val	Leu	Met	Gly	Ser	Ala	
			1045						1050					1055		
Glu	Ile	Thr	Gly	Leu	Gly	Ala	Ile	Arg	Glu	Asp	Asn	Asn	Ile	Thr	Leu	
		1060					1065						1070			
Lys	Leu	Asp	Arg	Cys	Asn	Asn	Asn	Asn	Gln	Tyr	Val	Ser	Ile	Asp	Lys	
		1075					1080						1085			
Phe	Arg	Ile	Phe	Cys	Lys	Ala	Leu	Asn	Pro	Lys	Glu	Ile	Glu	Lys	Leu	
	1090					1095					1100					
Tyr	Thr	Ser	Tyr	Leu	Ser	Ile	Thr	Phe	Leu	Arg	Asp	Phe	Trp	Gly	Asn	

Li *et al.*, Degradable Clostridial Toxins

1105		1110		1115		1120									
Pro	Leu	Arg	Tyr	Asp	Thr	Glu	Tyr	Tyr	Leu	Ile	Pro	Val	Ala	Ser	Ser
				1125					1130					1135	
Ser	Lys	Asp	Val	Gln	Leu	Lys	Asn	Ile	Thr	Asp	Tyr	Met	Tyr	Leu	Thr
			1140					1145					1150		
Asn	Ala	Pro	Ser	Tyr	Thr	Asn	Gly	Lys	Leu	Asn	Ile	Tyr	Tyr	Arg	Arg
		1155					1160					1165			
Leu	Tyr	Asn	Gly	Leu	Lys	Phe	Ile	Ile	Lys	Arg	Tyr	Thr	Pro	Asn	Asn
	1170				1175					1180					
Glu	Ile	Asp	Ser	Phe	Val	Lys	Ser	Gly	Asp	Phe	Ile	Lys	Leu	Tyr	Val
1185				1190						1195				1200	
Ser	Tyr	Asn	Asn	Asn	Glu	His	Ile	Val	Gly	Tyr	Pro	Lys	Asp	Gly	Asn
			1205						1210				1215		
Ala	Phe	Asn	Asn	Leu	Asp	Arg	Ile	Leu	Arg	Val	Gly	Tyr	Asn	Ala	Pro
		1220						1225					1230		
Gly	Ile	Pro	Leu	Tyr	Lys	Lys	Met	Glu	Ala	Val	Lys	Leu	Arg	Asp	Leu
	1235						1240					1245			
Lys	Thr	Tyr	Ser	Val	Gln	Leu	Lys	Leu	Tyr	Asp	Asp	Lys	Asn	Ala	Ser
	1250				1255					1260					
Leu	Gly	Leu	Val	Gly	Thr	His	Asn	Gly	Gln	Ile	Gly	Asn	Asp	Pro	Asn
1265				1270					1275					1280	
Arg	Asp	Ile	Leu	Ile	Ala	Ser	Asn	Trp	Tyr	Phe	Asn	His	Leu	Lys	Asp
			1285					1290					1295		
Lys	Ile	Leu	Gly	Cys	Asp	Trp	Tyr	Phe	Val	Pro	Thr	Asp	Glu	Gly	Trp
		1300						1305					1310		
Thr	Asn	Asp													
		1315													

&lt;210&gt; 9

&lt;211&gt; 425

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(425)

&lt;223&gt; PAR1

&lt;400&gt; 9

Met	Gly	Pro	Arg	Arg	Leu	Leu	Leu	Val	Ala	Ala	Cys	Phe	Ser	Leu	Cys
1				5					10					15	
Gly	Pro	Leu	Leu	Ser	Ala	Arg	Thr	Arg	Ala	Arg	Arg	Pro	Glu	Ser	Lys
		20						25					30		
Ala	Thr	Asn	Ala	Thr	Leu	Asp	Pro	Arg	Ser	Phe	Leu	Leu	Arg	Asn	Pro
		35					40					45			
Asn	Asp	Lys	Tyr	Glu	Pro	Phe	Trp	Glu	Asp	Glu	Glu	Lys	Asn	Glu	Ser
	50					55				60					
Gly	Leu	Thr	Glu	Tyr	Arg	Leu	Val	Ser	Ile	Asn	Lys	Ser	Ser	Pro	Leu
65					70				75					80	
Gln	Lys	Gln	Leu	Pro	Ala	Phe	Ile	Ser	Glu	Asp	Ala	Ser	Gly	Tyr	Leu
			85					90					95		
Thr	Ser	Ser	Trp	Leu	Thr	Leu	Phe	Val	Pro	Ser	Val	Tyr	Thr	Gly	Val
		100						105					110		
Phe	Val	Val	Ser	Leu	Pro	Leu	Asn	Ile	Met	Ala	Ile	Val	Val	Phe	Ile
	115						120					125			
Leu	Lys	Met	Lys	Val	Lys	Lys	Pro	Ala	Val	Val	Tyr	Met	Leu	His	Leu
	130					135					140				
Ala	Thr	Ala	Asp	Val	Leu	Phe	Val	Ser	Val	Leu	Pro	Phe	Lys	Ile	Ser

## Li et al., Degradable Clostridial Toxins

```

145          150          155          160
Tyr Tyr Phe Ser Gly Ser Asp Trp Gln Phe Gly Ser Glu Leu Cys Arg
          165          170          175
Phe Val Thr Ala Ala Phe Tyr Cys Asn Met Tyr Ala Ser Ile Leu Leu
          180          185          190
Met Thr Val Ile Ser Ile Asp Arg Phe Leu Ala Val Val Tyr Pro Met
          195          200          205
Gln Ser Leu Ser Trp Arg Thr Leu Gly Arg Ala Ser Phe Thr Cys Leu
          210          215          220
Ala Ile Trp Ala Leu Ala Ile Ala Gly Val Val Pro Leu Leu Leu Lys
225          230          235          240
Glu Gln Thr Ile Gln Val Pro Gly Leu Asn Ile Thr Thr Cys His Asp
          245          250          255
Val Leu Asn Glu Thr Leu Leu Glu Gly Tyr Tyr Ala Tyr Tyr Phe Ser
          260          265          270
Ala Phe Ser Ala Val Phe Phe Phe Val Pro Leu Ile Ile Ser Thr Val
          275          280          285
Cys Tyr Val Ser Ile Ile Arg Cys Leu Ser Ser Ser Ala Val Ala Asn
          290          295          300
Arg Ser Lys Lys Ser Arg Ala Leu Phe Leu Ser Ala Ala Val Phe Cys
305          310          315          320
Ile Phe Ile Ile Cys Phe Gly Pro Thr Asn Val Leu Leu Ile Ala His
          325          330          335
Tyr Ser Phe Leu Ser His Thr Ser Thr Thr Glu Ala Ala Tyr Phe Ala
          340          345          350
Tyr Leu Leu Cys Val Cys Val Ser Ser Ile Ser Cys Cys Ile Asp Pro
          355          360          365
Leu Ile Tyr Tyr Tyr Ala Ser Ser Glu Cys Gln Arg Tyr Val Tyr Ser
          370          375          380
Ile Leu Cys Cys Lys Glu Ser Ser Asp Pro Ser Ser Tyr Asn Ser Ser
385          390          395          400
Gly Gln Leu Met Ala Ser Lys Met Asp Thr Cys Ser Ser Asn Leu Asn
          405          410          415
Asn Ser Ile Tyr Lys Lys Leu Leu Thr
          420          425

```

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<210> 10
<211> 397
<212> PRT
<213> Homo sapiens

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<220>
<221> PEPTIDE
<222> (1)...(397)
<223> PAR2

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```

<400> 10
Met Arg Ser Pro Ser Ala Ala Trp Leu Leu Gly Ala Ala Ile Leu Leu
 1          5          10          15
Ala Ala Ser Leu Ser Cys Ser Gly Thr Ile Gln Gly Thr Asn Arg Ser
          20          25          30
Ser Lys Gly Arg Ser Leu Ile Gly Lys Val Asp Gly Thr Ser His Val
          35          40          45
Thr Gly Lys Gly Val Thr Val Glu Thr Val Phe Ser Val Asp Glu Phe
          50          55          60
Ser Ala Ser Val Leu Thr Gly Lys Leu Thr Thr Val Phe Leu Pro Ile
65          70          75          80
Val Tyr Thr Ile Val Phe Val Val Gly Leu Pro Ser Asn Gly Met Ala

```

## Li et al., Degradable Clostridial Toxins

														85			90					95		
Leu	Trp	Val	Phe	Leu	Phe	Arg	Thr	Lys	Lys	Lys	His	Pro	Ala	Val	Ile									
			100				105						110											
Tyr	Met	Ala	Asn	Leu	Ala	Leu	Ala	Asp	Leu	Leu	Ser	Val	Ile	Trp	Phe									
			115				120						125											
Pro	Leu	Lys	Ile	Ala	Tyr	His	Ile	His	Gly	Asn	Asn	Trp	Ile	Tyr	Gly									
			130				135						140											
Glu	Ala	Leu	Cys	Asn	Val	Leu	Ile	Gly	Phe	Phe	Tyr	Gly	Asn	Met	Tyr									
145						150						155			160									
Cys	Ser	Ile	Leu	Phe	Met	Thr	Cys	Leu	Ser	Val	Gln	Arg	Tyr	Trp	Val									
			165						170						175									
Ile	Val	Asn	Pro	Met	Gly	His	Ser	Arg	Lys	Lys	Ala	Asn	Ile	Ala	Ile									
			180						185						190									
Gly	Ile	Ser	Leu	Ala	Ile	Trp	Leu	Leu	Ile	Leu	Leu	Val	Thr	Ile	Pro									
			195						200						205									
Leu	Tyr	Val	Val	Lys	Gln	Thr	Ile	Phe	Ile	Pro	Ala	Leu	Asn	Ile	Thr									
210						215						220												
Thr	Cys	His	Asp	Val	Leu	Pro	Glu	Gln	Leu	Leu	Val	Gly	Asp	Met	Phe									
225						230						235			240									
Asn	Tyr	Phe	Leu	Ser	Leu	Ala	Ile	Gly	Val	Phe	Leu	Phe	Pro	Ala	Phe									
			245						250						255									
Leu	Thr	Ala	Ser	Ala	Tyr	Val	Leu	Met	Ile	Arg	Met	Leu	Arg	Ser	Ser									
			260						265						270									
Ala	Met	Asp	Glu	Asn	Ser	Glu	Lys	Lys	Arg	Lys	Arg	Ala	Ile	Lys	Leu									
			275						280						285									
Ile	Val	Thr	Val	Leu	Ala	Met	Tyr	Leu	Ile	Cys	Phe	Thr	Pro	Ser	Asn									
290						295						300												
Leu	Leu	Leu	Val	Val	His	Tyr	Phe	Leu	Ile	Lys	Ser	Gln	Gly	Gln	Ser									
305						310						315			320									
His	Val	Tyr	Ala	Leu	Tyr	Ile	Val	Ala	Leu	Cys	Leu	Ser	Thr	Leu	Asn									
			325						330						335									
Ser	Cys	Ile	Asp	Pro	Phe	Val	Tyr	Tyr	Phe	Val	Ser	His	Asp	Phe	Arg									
			340						345						350									
Asp	His	Ala	Lys	Asn	Ala	Leu	Leu	Cys	Arg	Ser	Val	Arg	Thr	Val	Lys									
			355						360						365									
Gln	Met	Gln	Val	Ser	Leu	Thr	Ser	Lys	Lys	His	Ser	Arg	Lys	Ser	Ser									
370						375						380												
Ser	Tyr	Ser	Ser	Ser	Ser	Thr	Thr	Val	Lys	Thr	Ser	Tyr												
385			390						395															

```
<210> 11
<211> 374
<212> PRT
<213> Homo sapiens
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```
<220>
<221> PEPTIDE
<222> (1) ... (374)
<223> PAR3
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<400> 11  
Met Lys Ala Leu Ile Phe Ala Ala Ala Gly Leu Leu Leu Leu Leu Pro  
1 5 10 15  
Thr Phe Cys Gln Ser Gly Met Glu Asn Asp Thr Asn Asn Leu Ala Lys  
20 25 30  
Pro Thr Leu Pro Ile Lys Thr Phe Arg Gly Ala Pro Pro Asn Ser Phe  
35 40 45  
Glu Glu Phe Pro Phe Ser Ala Leu Glu Gly Trp Thr Gly Ala Thr Ile

Li *et al.*, Degradable Clostridial Toxins

50		55		60											
Thr	Val	Lys	Ile	Lys	Cys	Pro	Glu	Glu	Ser	Ala	Ser	His	Leu	His	Val
65					70					75					80
Lys	Asn	Ala	Thr	Met	Gly	Tyr	Leu	Thr	Ser	Ser	Leu	Ser	Thr	Lys	Leu
				85					90					95	
Ile	Pro	Ala	Ile	Tyr	Leu	Leu	Val	Phe	Val	Val	Gly	Val	Pro	Ala	Asn
			100					105					110		
Ala	Val	Thr	Leu	Trp	Met	Leu	Phe	Phe	Arg	Thr	Arg	Ser	Ile	Cys	Thr
	115						120					125			
Thr	Val	Phe	Tyr	Thr	Asn	Leu	Ala	Ile	Ala	Asp	Phe	Leu	Phe	Cys	Val
130					135						140				
Thr	Leu	Pro	Phe	Lys	Ile	Ala	Tyr	His	Leu	Asn	Gly	Asn	Asn	Trp	Val
145				150					155					160	
Phe	Gly	Glu	Val	Leu	Cys	Arg	Ala	Thr	Thr	Val	Ile	Phe	Tyr	Gly	Asn
			165					170						175	
Met	Tyr	Cys	Ser	Ile	Leu	Leu	Leu	Ala	Cys	Ile	Ser	Ile	Asn	Arg	Tyr
	180						185					190			
Leu	Ala	Ile	Val	His	Pro	Phe	Thr	Tyr	Arg	Gly	Leu	Pro	Lys	His	Thr
	195						200					205			
Tyr	Ala	Leu	Val	Thr	Cys	Gly	Leu	Val	Trp	Ala	Thr	Val	Phe	Leu	Tyr
210					215					220					
Met	Leu	Pro	Phe	Phe	Ile	Leu	Lys	Gln	Glu	Tyr	Tyr	Leu	Val	Gln	Pro
225				230					235					240	
Asp	Ile	Thr	Thr	Cys	His	Asp	Val	His	Asn	Thr	Cys	Glu	Ser	Ser	Ser
			245					250						255	
Pro	Phe	Gln	Leu	Tyr	Tyr	Phe	Ile	Ser	Leu	Ala	Phe	Phe	Gly	Phe	Leu
	260						265						270		
Ile	Pro	Phe	Val	Leu	Ile	Ile	Tyr	Cys	Tyr	Ala	Ala	Ile	Ile	Arg	Thr
	275						280					285			
Leu	Asn	Ala	Tyr	Asp	His	Arg	Trp	Leu	Trp	Tyr	Val	Lys	Ala	Ser	Leu
290				295							300				
Leu	Ile	Leu	Val	Ile	Phe	Thr	Ile	Cys	Phe	Ala	Pro	Ser	Asn	Ile	Ile
305				310					315					320	
Leu	Ile	Ile	His	His	Ala	Asn	Tyr	Tyr	Tyr	Asn	Asn	Thr	Asp	Gly	Leu
			325					330						335	
Tyr	Phe	Ile	Tyr	Leu	Ile	Ala	Leu	Cys	Leu	Gly	Ser	Leu	Asn	Ser	Cys
	340						345					350			
Leu	Asp	Pro	Phe	Leu	Tyr	Phe	Leu	Met	Ser	Lys	Thr	Arg	Asn	His	Ser
	355						360					365			
Thr	Ala	Tyr	Leu	Thr	Lys										
370															

&lt;210&gt; 12

&lt;211&gt; 385

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(385)

&lt;223&gt; PAR4

&lt;400&gt; 12

Met	Trp	Gly	Arg	Leu	Leu	Leu	Trp	Pro	Leu	Val	Leu	Gly	Phe	Ser	Leu
1				5				10					15		
Ser	Gly	Gly	Thr	Gln	Thr	Pro	Ser	Val	Tyr	Asp	Glu	Ser	Gly	Ser	Thr
			20				25						30		
Gly	Gly	Gly	Asp	Asp	Ser	Thr	Pro	Ser	Ile	Leu	Pro	Ala	Pro	Arg	Gly

Li *et al.*, Degradable Clostridial Toxins

		35					40					45			
Tyr	Pro	Gly	Gln	Val	Cys	Ala	Asn	Asp	Ser	Asp	Thr	Leu	Glu	Leu	Pro
	50					55					60				
Asp	Ser	Ser	Arg	Ala	Leu	Leu	Leu	Gly	Trp	Val	Pro	Thr	Arg	Leu	Val
65					70					75					80
Pro	Ala	Leu	Tyr	Gly	Leu	Val	Leu	Val	Val	Gly	Leu	Pro	Ala	Asn	Gly
				85					90					95	
Leu	Ala	Leu	Trp	Val	Leu	Ala	Thr	Gln	Ala	Pro	Arg	Leu	Pro	Ser	Thr
			100					105					110		
Met	Leu	Leu	Met	Asn	Leu	Ala	Thr	Ala	Asp	Leu	Leu	Leu	Ala	Leu	Ala
		115					120					125			
Leu	Pro	Pro	Arg	Ile	Ala	Tyr	His	Leu	Arg	Gly	Gln	Arg	Trp	Pro	Phe
	130					135					140				
Gly	Glu	Ala	Ala	Cys	Arg	Leu	Ala	Thr	Ala	Ala	Leu	Tyr	Gly	His	Met
145					150					155					160
Tyr	Gly	Ser	Val	Leu	Leu	Leu	Ala	Ala	Val	Ser	Leu	Asp	Arg	Tyr	Leu
				165					170					175	
Ala	Leu	Val	His	Pro	Leu	Arg	Ala	Arg	Ala	Leu	Arg	Gly	Arg	Arg	Leu
			180					185					190		
Ala	Leu	Gly	Leu	Cys	Met	Ala	Ala	Trp	Leu	Met	Ala	Ala	Ala	Leu	Ala
		195					200					205			
Leu	Pro	Leu	Thr	Leu	Gln	Arg	Gln	Thr	Phe	Arg	Leu	Ala	Arg	Ser	Asp
	210					215					220				
Arg	Val	Leu	Cys	His	Asp	Ala	Leu	Pro	Leu	Asp	Ala	Gln	Ala	Ser	His
225					230					235					240
Trp	Gln	Pro	Ala	Phe	Thr	Cys	Leu	Ala	Leu	Leu	Gly	Cys	Phe	Leu	Pro
				245					250					255	
Leu	Leu	Ala	Met	Leu	Leu	Cys	Tyr	Gly	Ala	Thr	Leu	His	Thr	Leu	Ala
			260					265					270		
Ala	Ser	Gly	Arg	Arg	Tyr	Gly	His	Ala	Leu	Arg	Leu	Thr	Ala	Val	Val
		275					280					285			
Leu	Ala	Ser	Ala	Val	Ala	Phe	Phe	Val	Pro	Ser	Asn	Leu	Leu	Leu	Leu
	290					295					300				
Leu	His	Tyr	Ser	Asp	Pro	Ser	Pro	Ser	Ala	Trp	Gly	Asn	Leu	Tyr	Gly
305					310					315					320
Ala	Tyr	Val	Pro	Ser	Leu	Ala	Leu	Ser	Thr	Leu	Asn	Ser	Cys	Val	Asp
				325					330					335	
Pro	Phe	Ile	Tyr	Tyr	Tyr	Val	Ser	Ala	Glu	Phe	Arg	Asp	Lys	Val	Arg
			340					345					350		
Ala	Gly	Leu	Phe	Gln	Arg	Ser	Pro	Gly	Asp	Thr	Val	Ala	Ser	Lys	Ala
		355					360					365			
Ser	Ala	Glu	Gly	Gly	Ser	Arg	Gly	Met	Gly	Thr	His	Ser	Ser	Leu	Leu
	370					375					380				</

<210> 13

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1) ... (6)

<223> Hexapeptide comprising the tethered ligand of PAR1

<400> 13

Ser Phe Phe Leu Arg Asn



Li *et al.*, Degradable Clostridial Toxins

1

5

&lt;210&gt; 14

&lt;211&gt; 6

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR1

&lt;400&gt; 14

Ser Phe Phe Leu Arg Asn

1

5

&lt;210&gt; 15

&lt;211&gt; 6

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR1

&lt;400&gt; 15

Thr Phe Leu Leu Arg Asn

1

5

&lt;210&gt; 16

&lt;211&gt; 6

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR1

&lt;400&gt; 16

Gly Phe Pro Gly Lys Phe

1

5

&lt;210&gt; 17

&lt;211&gt; 6

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(6)

Li *et al.*, Degradable Clostridial Toxins

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR1

<400> 17

Gly Tyr Pro Ala Lys Phe  
1 5

<210> 18

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR1

<400> 18

Gly Tyr Pro Leu Lys Phe  
1 5

<210> 19

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR1

<400> 19

Gly Tyr Pro Ile Lys Phe  
1 5

<210> 20

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR1

<221> MOD\_RES

<222> (2)...(2)

<223> Xaa is parafluoro-phenylalanine (F).

<400> 20

Gly Xaa Pro Gly Lys Phe  
1 5

Li *et al.*, Degradable Clostridial Toxins

<210> 21  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR1

<221> MOD\_RES  
<222> (4)...(4)  
<223> Xaa is cyclohexylalanine (Cha).

<400> 21  
Gly Tyr Pro Xaa Lys Phe  
1 5

<210> 22  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR1

<221> MOD\_RES  
<222> (2)...(2)  
<223> Xaa is parafluoro-phenylalanine (F).

<221> MOD\_RES  
<222> (3)...(3)  
<223> Xaa is cyclohexylalanine (Cha).

<221> MOD\_RES  
<222> (4)...(4)  
<223> Xaa is cyclohexylalanine (Cha).

<400> 22  
Ser Xaa Xaa Xaa Arg Lys  
1 5

<210> 23  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)

Li *et al.*, Degradable Clostridial Toxins

<223> Variant of hexapeptide comprising the tethered ligand of PAR1

<221> MOD\_RES

<222> (2)...(2)

<223> Xaa is parafluoro-phenylalanine (F).

<221> MOD\_RES

<222> (3)...(3)

<223> Xaa is cyclohexylalanine (Cha).

<221> MOD\_RES

<222> (4)...(4)

<223> Xaa is cyclohexylalanine (Cha).

<221> MOD\_RES

<222> (5)...(5)

<223> Xaa is homoarginine (homoR).

<400> 23

Ser Xaa Xaa Xaa Xaa Lys

1

5

<210> 24

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Hexapeptide comprising the tethered ligand of PAR2

<400> 24

Ser Leu Ile Gly Lys Val

1

5

<210> 25

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered ligand of PAR2

<400> 25

Ser Leu Ile Gly Arg Leu

1

5

<210> 26

<211> 6

<212> PRT

<213> Artificial Sequence

Li *et al.*, Degradable Clostridial Toxins

<223> Variant of hexapeptide comprising the tethered ligand of PAR1

<221> MOD\_RES  
<222> (2)...(2)  
<223> Xaa is parafluoro-phenylalanine (F).

<221> MOD\_RES  
<222> (3)...(3)  
<223> Xaa is cyclohexylalanine (Cha).

<221> MOD\_RES  
<222> (4)...(4)  
<223> Xaa is cyclohexylalanine (Cha).

<221> MOD\_RES  
<222> (5)...(5)  
<223> Xaa is homoarginine (homoR).

<400> 23  
Ser Xaa Xaa Xaa Xaa Lys  
1 5

<210> 24  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Hexapeptide comprising the tethered ligand of PAR2

<400> 24  
Ser Leu Ile Gly Lys Val  
1 5

<210> 25  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered ligand of PAR2

<400> 25  
Ser Leu Ile Gly Arg Leu  
1 5

<210> 26  
<211> 6  
<212> PRT  
<213> Artificial Sequence

Li *et al.*, Degradable Clostridial Toxins

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Hexapeptide comprising the tethered ligand of PAR3

<400> 26  
Thr Phe Arg Gly Ala Pro  
1 5

<210> 27  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR3

<400> 27  
Ser Phe Asn Gly Gly Pro  
1 5

<210> 28  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Hexapeptide comprising the tethered ligand of PAR4

<400> 28  
Gly Tyr Pro Gly Gln Val  
1 5

<210> 29  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 29  
Ala Tyr Pro Gly Lys Phe  
1 5

<210> 30

Li *et al.*, Degradable Clostridial Toxins

<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 30  
Thr Tyr Pro Gly Lys Phe  
1 5

<210> 31  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 31  
Gly Tyr Pro Gly Lys Tyr  
1 5

<210> 32  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 32  
Gly Tyr Pro Gly Lys Trp  
1 5

<210> 33  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 33

Li *et al.*, Degradable Clostridial Toxins

Gly Tyr Pro Gly Lys Lys  
1 5

<210> 34  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 34  
Gly Tyr Pro Gly Lys Phe  
1 5

<210> 35  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 35  
Gly Tyr Pro Gly Arg Phe  
1 5

<210> 36  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 36  
Gly Tyr Pro Gly Phe Lys  
1 5

<210> 37  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE



Li *et al.*, Degradable Clostridial Toxins

<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 37  
Gly Tyr Pro Ala Lys Phe  
1 5

<210> 38  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 38  
Gly Phe Pro Gly Lys Phe  
1 5

<210> 39  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 39  
Gly Phe Pro Gly Lys Pro  
1 5

<210> 40  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 40  
Ser Tyr Pro Gly Lys Phe  
1 5

<210> 41  
<211> 6

Li *et al.*, Degradable Clostridial Toxins

<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 41  
Ser Tyr Pro Ala Lys Phe  
1 5

<210> 42  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 42  
Ser Tyr Pro Gly Arg Phe  
1 5

<210> 43  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<400> 43  
Ser Tyr Ala Gly Lys Phe  
1 5

<210> 44  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(6)  
<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<221> MOD\_RES  
<222> (5)...(5)

Li *et al.*, Degradable Clostridial Toxins

<223> Xaa is ornithine (Orn).

<400> 44

Gly Tyr Pro Gly Xaa Phe  
1 5

<210> 45

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<221> MOD\_RES

<222> (2)...(2)

<223> Xaa is parafluoro-phenylalanine (F).

<400> 45

Gly Xaa Pro Gly Lys Phe  
1 5

<210> 46

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<221> MOD\_RES

<222> (5)...(5)

<223> Xaa is homoarginine (homoR).

<400> 46

Gly Tyr Pro Gly Xaa Phe  
1 5

<210> 47

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4

<221> MOD\_RES

Li *et al.*, Degradable Clostridial Toxins

&lt;222&gt; (5)...(5)

&lt;223&gt; Xaa is homoarginine (homoR).

&lt;400&gt; 47

Ser Tyr Pro Gly Xaa Phe  
1 5

&lt;210&gt; 48

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(5)

&lt;223&gt; Flexible peptide spacer

&lt;400&gt; 48

Gly Gly Gly Gly Ser  
1 5

&lt;210&gt; 49

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(5)

&lt;223&gt; Flexible peptide spacer

&lt;400&gt; 49

Glu Ala Ala Ala Lys  
1 5

&lt;210&gt; 50

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1)...(5)

&lt;223&gt; Bovine enterokinase protease cleavage site.

&lt;400&gt; 50

Asp Asp Asp Asp Lys  
1 5

&lt;210&gt; 51

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

## Li et al., Degradable Clostridial Toxins

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Tobacco Etch Virus protease cleavage site.

<400> 51  
Glu Asn Leu Tyr Phe Gln Gly  
1 5

<210> 52  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Tobacco Etch Virus protease cleavage site.

<400> 52  
Glu Asn Leu Tyr Phe Gln Ser  
1 5

<210> 53  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Tobacco Etch Virus protease cleavage site.

<400> 53  
Glu Asn Ile Tyr Thr Gln Gly  
1 5

<210> 54  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Tobacco Etch Virus protease cleavage site.

<400> 54  
Glu Asn Ile Tyr Thr Gln Ser  
1 5

<210> 55  
<211> 7  
<212> PRT  
<213> Artificial Sequence

Li *et al.*, Degradable Clostridial Toxins

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Tobacco Etch Virus protease cleavage site.

<400> 55  
Glu Asn Ile Tyr Leu Gln Gly  
1 5

<210> 56  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Tobacco Etch Virus protease cleavage site.

<400> 56  
Glu Asn Ile Tyr Leu Gln Ser  
1 5

<210> 57  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Tobacco Etch Virus protease cleavage site.

<400> 57  
Glu Asn Val Tyr Phe Gln Gly  
1 5

<210> 58  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Tobacco Etch Virus protease cleavage site.

<400> 58  
Glu Asn Val Tyr Ser Gln Ser  
1 5

<210> 59  
<211> 7  
<212> PRT

Li *et al.*, Degradable Clostridial Toxins

<213> Artificial Sequence

<220>

<221> SITE

<222> (1)...(7)

<223> Tobacco Etch Virus protease cleavage site.

<400> 59

Glu Asn Val Tyr Ser Gln Gly  
1 5

<210> 60

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> SITE

<222> (0)...(0)

<223> Tobacco Etch Virus protease cleavage site.

<400> 60

Glu Asn Val Tyr Ser Gln Ser  
1 5

<210> 61

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> SITE

<222> (1)...(7)

<223> Human Rhinovirus 3C protease cleavage site.

<400> 61

Glu Ala Leu Phe Gln Gly Pro  
1 5

<210> 62

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> SITE

<222> (1)...(7)

<223> Human Rhinovirus 3C protease cleavage site.

<400> 62

Glu Val Leu Phe Gln Gly Pro  
1 5

<210> 63

<211> 7

## Li et al., Degradable Clostridial Toxins

<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Human Rhinovirus 3C protease cleavage site.

<400> 63  
Glu Leu Leu Phe Gln Gly Pro  
1 5

<210> 64  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Human Rhinovirus 3C protease cleavage site.

<400> 64  
Asp Ala Leu Phe Gln Gly Pro  
1 5

<210> 65  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(7)  
<223> Human Rhinovirus 3C protease cleavage site.

<400> 65  
Asp Val Leu Phe Gln Gly Pro  
1 5

<210> 66  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (0)...(0)  
<223> Human Rhinovirus 3C protease cleavage site.

<400> 66  
Asp Leu Leu Phe Gln Gly Pro  
1 5



## Li et al.; Degradable Clostridial Toxins

<210> 67  
 <211> 98  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> SITE  
 <222> (1)...(98)  
 <223> SUMO/ULP-1 protease cleavage site.

<400> 67  
 Met Ala Asp Ser Glu Val Asn Gln Glu Ala Lys Pro Glu Val Lys Pro  
 1 5 10 15  
 Glu Val Lys Pro Glu Thr His Ile Asn Leu Lys Val Ser Asp Gly Ser  
 20 25 30  
 Ser Glu Ile Phe Phe Lys Ile Lys Lys Thr Thr Pro Leu Arg Arg Leu  
 35 40 45  
 Met Glu Ala Phe Ala Lys Arg Gln Gly Lys Glu Met Asp Ser Leu Arg  
 50 55 60  
 Phe Leu Tyr Asp Gly Ile Arg Ile Gln Ala Asp Gln Thr Pro Glu Asp  
 65 70 75 80  
 Leu Asp Met Glu Asp Asn Asp Ile Ile Glu Ala His Arg Glu Gln Ile  
 85 90 95  
 Gly Gly

<210> 68  
 <211> 4  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> SITE  
 <222> (1)...(4)  
 <223> Thrombin protease cleavage site.

<400> 68  
 Gly Val Arg Gly  
 1

<210> 69  
 <211> 4  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> SITE  
 <222> (1)...(4)  
 <223> Thrombin protease cleavage site.

<400> 69  
 Ser Ala Arg Gly  
 1

<210> 70  
 <211> 4

Li *et al.*, Degradable Clostridial Toxins

<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(4)  
<223> Thrombin protease cleavage site.

<400> 70  
Ser Leu Arg Gly  
1

<210> 71  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(4)  
<223> Thrombin protease cleavage site.

<400> 71  
Asp Gly Arg Ile  
1

<210> 72  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(4)  
<223> Thrombin protease cleavage site.

<400> 72  
Gln Gly Lys Ile  
1

<210> 73  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(6)  
<223> Thrombin protease cleavage site.

<400> 73  
Leu Val Pro Arg Gly Ser  
1 5

<210> 74

Li *et al.*, Degradable Clostridial Toxins

<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(6)  
<223> Thrombin protease cleavage site.

<400> 74  
Leu Val Pro Lys Gly Ser  
1 5

<210> 75  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(6)  
<223> Thrombin protease cleavage site.

<400> 75  
Phe Ile Pro Arg Thr Phe  
1 5

<210> 76  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(6)  
<223> Thrombin protease cleavage site.

<400> 76  
Val Leu Pro Arg Ser Phe  
1 5

<210> 77  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(6)  
<223> Thrombin protease cleavage site.

<400> 77  
Ile Val Pro Arg Ser Phe  
1 5

Li *et al.*, Degradable Clostridial Toxins

<210> 78  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(6)  
<223> Thrombin protease cleavage site.

<400> 78  
Ile Val Pro Arg Gly Tyr  
1 5

<210> 79  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(6)  
<223> Thrombin protease cleavage site.

<400> 79  
Val Val Pro Arg Gly Val  
1 5

<210> 80  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(6)  
<223> Thrombin protease cleavage site.

<400> 80  
Val Leu Pro Arg Leu Ile  
1 5

<210> 81  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> SITE  
<222> (1)...(6)  
<223> Thrombin protease cleavage site.

<400> 81  
Val Met Pro Arg Ser Leu  
1 5

Li *et al.*, Degradable Clostridial Toxins

<210> 82  
 <211> 6  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> SITE  
 <222> (1)...(6)  
 <223> Thrombin protease cleavage site.

<400> 82  
 Met Phe Pro Arg Ser Leu  
 1 5

<210> 83  
 <211> 4  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> SITE  
 <222> (1)...(4)  
 <223> Coagulation Factor Xa protease cleavage site.

<400> 83  
 Ile Asp Gly Arg  
 1

<210> 84  
 <211> 4  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> SITE  
 <222> (1)...(4)  
 <223> Coagulation Factor Xa protease cleavage site.

<400> 84  
 Ile Glu Gly Arg  
 1

<210> 85  
 <211> 1350  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> PEPTIDE  
 <222> (1)...(1350)  
 <223> BoNT/A-ED-PAR1-Thrombin

<400> 85  
 Met Gly Pro Arg Arg Leu Leu Leu Val Ala Ala Cys Phe Ser Leu Cys  
 1 5 10 15

## Li et al., Degradable Clostridial Toxins

Gly	Pro	Leu	Leu	Ser	Ala	Arg	Thr	Arg	Ala	Arg	Arg	Pro	Glu	Ser	Lys
			20					25					30		
Ala	Thr	Asn	Ala	Thr	Leu	Asp	Pro	Arg	Ser	Phe	Leu	Leu	Arg	Asn	Pro
		35					40					45			
Asn	Asp	Lys	Tyr	Glu	Pro	Phe	Pro	Phe	Val	Asn	Lys	Gln	Phe	Asn	Tyr
	50					55					60				
Lys	Asp	Pro	Val	Asn	Gly	Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn
65					70					75					80
Ala	Gly	Gln	Met	Gln	Pro	Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile
				85					90					95	
Trp	Val	Ile	Pro	Glu	Arg	Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp
			100					105					110		
Leu	Asn	Pro	Pro	Pro	Glu	Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp
		115					120					125			
Ser	Thr	Tyr	Leu	Ser	Thr	Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly
	130					135					140				
Val	Thr	Lys	Leu	Phe	Glu	Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met
145					150					155					160
Leu	Leu	Thr	Ser	Ile	Val	Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr
				165					170					175	
Ile	Asp	Thr	Glu	Leu	Lys	Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile
		180						185					190		
Gln	Pro	Asp	Gly	Ser	Tyr	Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile
		195					200					205			
Gly	Pro	Ser	Ala	Asp	Ile	Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His
	210					215					220				
Glu	Val	Leu	Asn	Leu	Thr	Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile
225					230					235					240
Arg	Phe	Ser	Pro	Asp	Phe	Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val
				245					250					255	
Asp	Thr	Asn	Pro	Leu	Leu	Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala
			260					265					270		
Val	Thr	Leu	Ala	His	Glu	Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly
		275					280					285			
Ile	Ala	Ile	Asn	Pro	Asn	Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr
	290					295					300				
Tyr	Glu	Met	Ser	Gly	Leu	Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe
305					310					315					320
Gly	Gly	His	Asp	Ala	Lys	Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe
				325					330					335	
Arg	Leu	Tyr	Tyr	Asn	Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	
		340					345					350			
Lys	Ala	Lys	Ser	Ile	Val	Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys
		355					360					365			
Asn	Val	Phe	Lys	Glu	Lys	Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys
	370					375					380				
Phe	Ser	Val	Asp	Lys	Leu	Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr
385					390					395					400
Glu	Ile	Tyr	Thr	Glu	Asp	Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn
				405					410					415	
Arg	Lys	Thr	Tyr	Leu	Asn	Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile
		420						425					430		
Val	Pro	Lys	Val	Asn	Tyr	Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn
		435					440					445			
Thr	Asn	Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn
	450					455					460				
Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr
465					470					475					480
Lys	Leu	Leu	Cys	Val	Arg	Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu

Li *et al.*, Degradable Clostridial Toxins

				485				490					495				
Asp	Lys	Gly	Tyr	Asn	Lys	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn		
			500					505					510				
Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp		
		515					520					525					
Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala		
	530					535					540						
Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe		
545				550					555						560		
Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser		
			565						570					575			
Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro		
			580					585					590				
Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu		
		595					600					605					
Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn		
	610					615					620						
Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe		
625					630					635					640		
Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met		
			645						650					655			
Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr		
		660						665					670				
Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile		
	675						680						685				
Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp		
	690					695					700						
Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu		
705					710					715					720		
Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val		
			725						730					735			
Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala		
			740					745					750				
Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val		
		755					760					765					
Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys		
	770					775					780						
Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile		
785					790					795					800		
Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile		
			805						810					815			
Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn		
			820					825					830				
Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser		
		835					840					845					
Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp		
	850					855					860						
Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn		
865					870					875					880		
Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn		
			885						890					895			
Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp		
		900						905					910				
Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile		
		915					920						925				
Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp		
	930					935						940					
Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe		
945					950					955					960		

Li *et al.*, Degradable Clostridial Toxins

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Asp Pro Ile Asp Lys Asn Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser
          965                      970                      975
Lys Ile Glu Val Ile Leu Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr
          980                      985                      990
Glu Asn Phe Ser Thr Ser Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn
          995                      1000                     1005
Ser Ile Ser Leu Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn
          1010                     1015                     1020
Asn Ser Gly Trp Lys Val Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr
          1025                     1030                     1035                     1040
Leu Gln Asp Thr Gln Glu Ile Lys Gln Arg Val Val Phe Lys Tyr Ser
          1045                     1050                     1055
Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr
          1060                     1065                     1070
Ile Thr Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg
          1075                     1080                     1085
Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser
          1090                     1095                     1100
Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr
          1105                     1110                     1115                     1120
Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys
          1125                     1130                     1135
Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys
          1140                     1145                     1150
Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu
          1155                     1160                     1165
Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile
          1170                     1175                     1180
Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr
          1185                     1190                     1195                     1200
Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile
          1205                     1210                     1215
Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp
          1220                     1225                     1230
Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala
          1235                     1240                     1245
Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu
          1250                     1255                     1260
Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys
          1265                     1270                     1275                     1280
Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn
          1285                     1290                     1295
Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile
          1300                     1305                     1310
Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser
          1315                     1320                     1325
Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly
          1330                     1335                     1340
Trp Gly Glu Arg Pro Leu
          1345                     1350

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&lt;210&gt; 86

&lt;211&gt; 1342

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE



## Li et al., Degradable Clostridial Toxins

&lt;222&gt; (1)...(1342)

&lt;223&gt; BoNT/A-ED-PAR1-Xa

&lt;400&gt; 86

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Met Gly Pro Arg Arg Leu Leu Leu Val Ala Ala Cys Phe Ser Leu Cys
 1           5           10           15
Gly Pro Leu Leu Ser Ala Arg Thr Arg Ala Arg Arg Pro Glu Ser Lys
           20           25           30
Ala Thr Asn Ala Thr Ile Glu Gly Arg Ser Phe Leu Leu Arg Asn Pro
 35           40           45
Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly Val Asp
 50           55           60
Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro Val Lys
 65           70           75           80
Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg Asp Thr
           85           90           95
Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu Ala Lys
           100          105          110
Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr Asp Asn
           115          120          125
Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu Arg Ile
           130          135          140
Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val Arg Gly
 145          150          155          160
Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys Val Ile
           165          170          175
Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr Arg Ser
           180          185          190
Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile Ile Gln
           195          200          205
Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr Arg Asn
           210          215          220
Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe Thr Phe
 225          230          235          240
Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu Gly Ala
           245          250          255
Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu Leu Ile
           260          265          270
His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn Arg Val
           275          280          285
Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu Glu Val
           290          295          300
Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys Phe Ile
 305          310          315          320
Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn Lys Phe
           325          330          335
Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val Gly Thr
           340          345          350
Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys Tyr Leu
           355          360          365
Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu Lys Phe
           370          375          380
Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp Asn Phe
 385          390          395          400
Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn Phe Asp
           405          410          415
Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr Thr Ile
           420          425          430
Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn Phe Asn

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## Li et al., Degradable Clostridial Toxins

	435					440					445				
Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn
	450					455					460				
Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	Gly	Ile
465					470					475					480
Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Asn	Lys	Ala	Leu
				485					490					495	
Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro
		500						505					510		
Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr
	515						520					525			
Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu
	530					535					540				
Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn
545					550					555					560
Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu
				565				570						575	
Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp
		580					585						590		
Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly
	595						600					605			
Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn
	610					615					620				
Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val
625					630					635					640
Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu
				645				650						655	
Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys
		660					665						670		
Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn
	675					680						685			
Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe
	690					695					700				
Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro
705					710					715					720
Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu
				725				730						735	
Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp
		740					745						750		
Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn
	755						760					765			
Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn
	770					775					780				
Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr
785					790					795					800
Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser
				805				810						815	
Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys
		820						825					830		
Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro
	835						840					845			
Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala
	850					855					860				
Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val
865					870					875					880
Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro
				885					890					895	
Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe
		900						905						910	

## Li et al., Degradable Clostridial Toxins

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Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn Leu Arg
    915                      920                      925
Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser Lys Ile
    930                      935                      940
Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn Gln Ile
    945                      950                      955                      960
Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn
    965                      970                      975
Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp
    980                      985                      990
Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn Glu Tyr
    995                      1000                      1005
Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu
    1010                      1015                      1020
Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu Ile Lys
    1025                      1030                      1035                      1040
Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr
    1045                      1050                      1055
Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn
    1060                      1065                      1070
Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser
    1075                      1080                      1085
Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp
    1090                      1095                      1100
Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu
    1105                      1110                      1115                      1120
Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn
    1125                      1130                      1135
Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln
    1140                      1145                      1150
Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr
    1155                      1160                      1165
Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly
    1170                      1175                      1180
Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu
    1185                      1190                      1195                      1200
Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys
    1205                      1210                      1215
Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val
    1220                      1225                      1230
Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val
    1235                      1240                      1245
Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser
    1250                      1255                      1260
Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys
    1265                      1270                      1275                      1280
Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile
    1285                      1290                      1295
Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp
    1300                      1305                      1310
Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp
    1315                      1320                      1325
Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
    1330                      1335                      1340

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&lt;210&gt; 87

&lt;211&gt; 1345

&lt;212&gt; PRT

Li *et al.*, Degradable Clostridial Toxins

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1345)

&lt;223&gt; BoNT/A-ED-PAR2-Trypsin

&lt;400&gt; 87

Met	Arg	Ser	Pro	Ser	Ala	Ala	Trp	Leu	Leu	Gly	Ala	Ala	Ile	Leu	Leu
1				5				10					15		
Ala	Ala	Ser	Leu	Ser	Cys	Ser	Gly	Thr	Ile	Gln	Gly	Thr	Asn	Arg	Ser
		20					25						30		
Ser	Lys	Gly	Arg	Ser	Leu	Ile	Gly	Lys	Val	Asp	Gly	Thr	Ser	His	Val
	35						40					45			
Thr	Gly	Pro	Phe	Val	Asn	Lys	Gln	Phe	Asn	Tyr	Lys	Asp	Pro	Val	Asn
	50				55						60				
Gly	Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn	Ala	Gly	Gln	Met	Gln
65					70					75					80
Pro	Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile	Trp	Val	Ile	Pro	Glu
				85					90					95	
Arg	Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro
			100					105					110		
Glu	Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser
	115						120					125			
Thr	Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe
	130					135					140				
Glu	Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile
145					150					155					160
Val	Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu
				165					170					175	
Lys	Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser
			180					185					190		
Tyr	Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp
	195						200					205			
Ile	Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu
	210					215					220				
Thr	Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp
225					230					235					240
Phe	Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu
				245					250					255	
Leu	Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His
		260						265					270		
Glu	Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro
	275						280					285			
Asn	Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly
	290					295					300				
Leu	Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala
305					310					315					320
Lys	Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr
				325					330					335	
Asn	Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile
				340				345					350		
Val	Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu
	355						360					365			
Lys	Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys
	370					375					380				
Leu	Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu
385					390					395					400
Asp	Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu

Li *et al.*, Degradable Clostridial Toxins

				405					410					415	
Asn	Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn
			420					425					430		
Tyr	Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala
			435					440					445		
Asn	Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys
			450					455				460			
Leu	Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val
465						470				475					480
Arg	Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Asp	Lys	Gly	Tyr	Asn
				485					490						495
Lys	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe
			500					505					510		
Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu
			515					520					525		
Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser
			530					535					540		
Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu
545						550				555					560
Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln
						565				570					575
Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr
			580					585					590		
Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe
			595					600					605		
Glu	His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala
			610					615					620		
Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val
625						630				635					640
Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val
						645				650					655
Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr
						660				665					670
Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro
						675				680					685
Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala
						690							700		
Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile
705						710				715					720
Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn
						725				730					735
Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn
						740				745					750
Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala
						755				760					765
Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala
						770				775					780
Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr
785						790				795					800
Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp
						805				810					815
Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn
						820				825					830
Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser
						835				840					845
Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu
						850				855					860
Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile
865						870				875					880

## Li et al., Degradable Clostridial Toxins

Gly Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr  
 885 890 895  
 Asp Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu  
 900 905 910  
 Ser Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu  
 915 920 925  
 Asn Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala  
 930 935 940  
 Ser Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys  
 945 950 955 960  
 Asn Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile  
 965 970 975  
 Leu Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr  
 980 985 990  
 Ser Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn  
 995 1000 1005  
 Asn Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys  
 1010 1015 1020  
 Val Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln  
 1025 1030 1035 1040  
 Glu Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile  
 1045 1050 1055  
 Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg  
 1060 1065 1070  
 Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys  
 1075 1080 1085  
 Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe  
 1090 1095 1100  
 Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr  
 1105 1110 1115 1120  
 Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu  
 1125 1130 1135  
 Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp  
 1140 1145 1150  
 Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro  
 1155 1160 1165  
 Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr  
 1170 1175 1180  
 Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn  
 1185 1190 1195 1200  
 Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser  
 1205 1210 1215  
 Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn  
 1220 1225 1230  
 Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln  
 1235 1240 1245  
 Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly  
 1250 1255 1260  
 Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile  
 1265 1270 1275 1280  
 Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile  
 1285 1290 1295  
 Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala  
 1300 1305 1310  
 Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly  
 1315 1320 1325  
 Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro  
 1330 1335 1340  
 Leu

Li *et al.*, Degradable Clostridial Toxins

1345

<210> 88  
 <211> 1337  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> PEPTIDE  
 <222> (1)...(1337)  
 <223> BoNT/A-ED-PAR2-Xa

<400> 88  
 Met Arg Ser Pro Ser Ala Ala Trp Leu Leu Gly Ala Ala Ile Leu Leu  
 1 5 10 15  
 Ala Ala Ser Leu Ser Cys Ser Gly Thr Ile Gln Gly Thr Asn Arg Ser  
 20 25 30  
 Ile Glu Gly Arg Ser Leu Ile Gly Lys Val Pro Phe Val Asn Lys Gln  
 35 40 45  
 Phe Asn Tyr Lys Asp Pro Val Asn Gly Val Asp Ile Ala Tyr Ile Lys  
 50 55 60  
 Ile Pro Asn Ala Gly Gln Met Gln Pro Val Lys Ala Phe Lys Ile His  
 65 70 75 80  
 Asn Lys Ile Trp Val Ile Pro Glu Arg Asp Thr Phe Thr Asn Pro Glu  
 85 90 95  
 Glu Gly Asp Leu Asn Pro Pro Pro Glu Ala Lys Gln Val Pro Val Ser  
 100 105 110  
 Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr Asp Asn Glu Lys Asp Asn Tyr  
 115 120 125  
 Leu Lys Gly Val Thr Lys Leu Phe Glu Arg Ile Tyr Ser Thr Asp Leu  
 130 135 140  
 Gly Arg Met Leu Leu Thr Ser Ile Val Arg Gly Ile Pro Phe Trp Gly  
 145 150 155 160  
 Gly Ser Thr Ile Asp Thr Glu Leu Lys Val Ile Asp Thr Asn Cys Ile  
 165 170 175  
 Asn Val Ile Gln Pro Asp Gly Ser Tyr Arg Ser Glu Glu Leu Asn Leu  
 180 185 190  
 Val Ile Ile Gly Pro Ser Ala Asp Ile Ile Gln Phe Glu Cys Lys Ser  
 195 200 205  
 Phe Gly His Glu Val Leu Asn Leu Thr Arg Asn Gly Tyr Gly Ser Thr  
 210 215 220  
 Gln Tyr Ile Arg Phe Ser Pro Asp Phe Thr Phe Gly Phe Glu Glu Ser  
 225 230 235 240  
 Leu Glu Val Asp Thr Asn Pro Leu Leu Gly Ala Gly Lys Phe Ala Thr  
 245 250 255  
 Asp Pro Ala Val Thr Leu Ala His Glu Leu Ile His Ala Gly His Arg  
 260 265 270  
 Leu Tyr Gly Ile Ala Ile Asn Pro Asn Arg Val Phe Lys Val Asn Thr  
 275 280 285  
 Asn Ala Tyr Tyr Glu Met Ser Gly Leu Glu Val Ser Phe Glu Glu Leu  
 290 295 300  
 Arg Thr Phe Gly Gly His Asp Ala Lys Phe Ile Asp Ser Leu Gln Glu  
 305 310 315 320  
 Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn Lys Phe Lys Asp Ile Ala Ser  
 325 330 335  
 Thr Leu Asn Lys Ala Lys Ser Ile Val Gly Thr Thr Ala Ser Leu Gln  
 340 345 350  
 Tyr Met Lys Asn Val Phe Lys Glu Lys Tyr Leu Leu Ser Glu Asp Thr

Li *et al.*, Degradable Clostridial Toxins

		355					360					365			
Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu	Lys	Phe	Asp	Lys	Leu	Tyr	Lys
	370					375					380				
Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp	Asn	Phe	Val	Lys	Phe	Phe	Lys
385					390					395					400
Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn	Phe	Asp	Lys	Ala	Val	Phe	Lys
				405					410					415	
Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr	Thr	Ile	Tyr	Asp	Gly	Phe	Asn
			420					425					430		
Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln	Asn	Thr	Glu
		435					440					445			
Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr	Gly	Leu	Phe
	450					455					460				
Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	Gly	Ile	Ile	Thr	Ser	Lys	Thr
465					470					475					480
Lys	Ser	Leu	Ile	Glu	Gly	Arg	Asn	Lys	Ala	Leu	Asn	Asp	Leu	Cys	Ile
				485					490					495	
Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe
			500					505					510		
Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile
		515					520						525		
Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr
	530					535					540				
Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn
545					550					555					560
Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu
				565					570					575	
Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe
			580					585						590	
His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	Ala
		595					600						605		
Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr
	610					615					620				
Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu
625					630					635					640
Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr
				645					650					655	
Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr
			660					665					670		
Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu
		675					680					685			
Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile
	690					695					700				
Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe
705					710					715					720
Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile
				725					730					735	
Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys
			740					745					750		
Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu
		755					760					765			
Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr
	770					775					780				
Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys
785					790					795					800
Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu
				805					810					815	
Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys
			820					825					830		



## Li et al., Degradable Clostridial Toxins

Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg
		835						840				845			
Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile
		850				855					860				
Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp
865					870					875					880
Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys
				885					890					895	
Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys
			900					905					910		
Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His
		915					920					925			
Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys
		930				935					940				
Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu
945					950					955					960
Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn
				965					970					975	
Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys
			980					985					990		
Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys
		995					1000					1005			
Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile
	1010					1015					1020				
Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe
1025					1030					1035					1040
Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile
				1045					1050					1055	
Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile
			1060					1065					1070		
Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile
		1075					1080					1085			
His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr
	1090					1095					1100				
His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu
1105					1110					1115					1120
Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly
				1125					1130					1135	
Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr
		1140						1145					1150		
Tyr	Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn
		1155					1160					1165			
Val	Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val
	1170					1175				</					

## Li et al., Degradable Clostridial Toxins

Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile  
                   1300                  1305                  1310  
 Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val  
                   1315                  1320                  1325  
 Asp Asp Gly Trp Gly Glu Arg Pro Leu  
                   1330                  1335

&lt;210&gt; 89

&lt;211&gt; 1347

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1347)

&lt;223&gt; BoNT/A-ED-PAR3-Thrombin

&lt;400&gt; 89

Met Lys Ala Leu Ile Phe Ala Ala Ala Gly Leu Leu Leu Leu Leu Pro  
   1                  5                  10                  15  
 Thr Phe Cys Gln Ser Gly Met Glu Asn Asp Thr Asn Asn Leu Ala Lys  
                   20                  25                  30  
 Pro Thr Leu Pro Ile Lys Thr Phe Arg Gly Ala Pro Pro Asn Ser Phe  
                   35                  40                  45  
 Glu Glu Phe Pro Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro  
                   50                  55                  60  
 Val Asn Gly Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln  
   65                  70                  75                  80  
 Met Gln Pro Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile  
                   85                  90                  95  
 Pro Glu Arg Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro  
                   100                  105                  110  
 Pro Pro Glu Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr  
                   115                  120                  125  
 Leu Ser Thr Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys  
                   130                  135                  140  
 Leu Phe Glu Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr  
   145                  150                  155                  160  
 Ser Ile Val Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr  
                   165                  170                  175  
 Glu Leu Lys Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp  
                   180                  185                  190  
 Gly Ser Tyr Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser  
                   195                  200                  205  
 Ala Asp Ile Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu  
                   210                  215                  220  
 Asn Leu Thr Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser  
   225                  230                  235                  240  
 Pro Asp Phe Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn  
                   245                  250                  255  
 Pro Leu Leu Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu  
                   260                  265                  270  
 Ala His Glu Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile  
                   275                  280                  285  
 Asn Pro Asn Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met  
                   290                  295                  300  
 Ser Gly Leu Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His  
   305                  310                  315                  320

## Li et al., Degradable Clostridial Toxins

Asp Ala Lys Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr  
 325 330 335  
 Tyr Tyr Asn Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys  
 340 345 350  
 Ser Ile Val Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe  
 355 360 365  
 Lys Glu Lys Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val  
 370 375 380  
 Asp Lys Leu Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr  
 385 390 395 400  
 Thr Glu Asp Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr  
 405 410 415  
 Tyr Leu Asn Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys  
 420 425 430  
 Val Asn Tyr Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu  
 435 440 445  
 Ala Ala Asn Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe  
 450 455 460  
 Thr Lys Leu Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu  
 465 470 475 480  
 Cys Val Arg Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly  
 485 490 495  
 Tyr Asn Lys Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp  
 500 505 510  
 Leu Phe Phe Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys  
 515 520 525  
 Gly Glu Glu Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn  
 530 535 540  
 Ile Ser Leu Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp  
 545 550 555 560  
 Asn Glu Pro Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile  
 565 570 575  
 Gly Gln Leu Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys  
 580 585 590  
 Lys Tyr Glu Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln  
 595 600 605  
 Glu Phe Glu His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn  
 610 615 620  
 Glu Ala Leu Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp  
 625 630 635 640  
 Tyr Val Lys Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly  
 645 650 655  
 Trp Val Glu Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val  
 660 665 670  
 Ser Thr Thr Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile  
 675 680 685  
 Gly Pro Ala Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val  
 690 695 700  
 Gly Ala Leu Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro  
 705 710 715 720  
 Glu Ile Ala Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile  
 725 730 735  
 Ala Asn Lys Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys  
 740 745 750  
 Arg Asn Glu Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp  
 755 760 765  
 Leu Ala Lys Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys  
 770 775 780  
 Glu Ala Leu Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr

Li *et al.*, Degradable Clostridial Toxins

785				790					795				800		
Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn
				805					810					815	
Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met
			820					825					830		
Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met
		835				840						845			
Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala
	850					855					860				
Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr
865				870						875					880
Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu
			885					890						895	
Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg
		900						905					910		
Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser
	915					920						925			
Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg
	930					935					940				
Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile
945				950						955					960
Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu
			965					970						975	
Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe
		980						985					990		
Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser
	995					1000						1005			
Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly
	1010					1015					1020				
Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp
1025				1030						1035					1040
Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile
			1045					1050						1055	
Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn
		1060				1065							1070		
Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp
	1075					1080						1085			
Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile
	1090					1095					1100				
Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile
1105				1110						1115					1120
Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys
			1125					1130						1135	
Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp
		1140						1145					1150		
Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr	Tyr	Met	Leu	Asn	Leu	Tyr
	1155					1160						1165			
Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn	Val	Gly	Ile	Arg	Gly	Tyr
	1170					1175					1180				
Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val	Met	Thr	Thr	Asn	Ile	Tyr
1185				1190						1195					1200
Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys	Phe	Ile	Ile	Lys	Lys	Tyr
			1205					1210						1215	
Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg	Asn	Asn	Asp	Arg	Val	Tyr
		1220						1225					1230		
Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr	Arg	Leu	Ala	Thr	Asn	Ala
	1235					1240						1245			
Ser	Gln	Ala	Gly	Val	Glu	Lys	Ile	Leu	Ser	Ala	Leu	Glu	Ile	Pro	Asp
	1250					1255					1260				

Li *et al.*, Degradable Clostridial Toxins

Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln  
 1265 1270 1275 1280  
 Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn  
 1285 1290 1295  
 Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu  
 1300 1305 1310  
 Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr  
 1315 1320 1325  
 Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu  
 1330 1335 1340  
 Arg Pro Leu  
 1345

&lt;210&gt; 90

&lt;211&gt; 1339

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1339)

&lt;223&gt; BoNT/A-ED-PAR3-Xa

&lt;400&gt; 90

Met Lys Ala Leu Ile Phe Ala Ala Ala Gly Leu Leu Leu Leu Leu Pro  
 1 5 10 15  
 Thr Phe Cys Gln Ser Gly Met Glu Asn Asp Thr Asn Asn Leu Ala Lys  
 20 25 30  
 Pro Thr Ile Glu Gly Arg Thr Phe Arg Gly Ala Pro Pro Phe Val Asn  
 35 40 45  
 Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly Val Asp Ile Ala Tyr  
 50 55 60  
 Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro Val Lys Ala Phe Lys  
 65 70 75 80  
 Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg Asp Thr Phe Thr Asn  
 85 90 95  
 Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu Ala Lys Gln Val Pro  
 100 105 110  
 Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr Asp Asn Glu Lys Asp  
 115 120 125  
 Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu Arg Ile Tyr Ser Thr  
 130 135 140  
 Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val Arg Gly Ile Pro Phe  
 145 150 155 160  
 Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys Val Ile Asp Thr Asn  
 165 170 175  
 Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr Arg Ser Glu Glu Leu  
 180 185 190  
 Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile Ile Gln Phe Glu Cys  
 195 200 205  
 Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr Arg Asn Gly Tyr Gly  
 210 215 220  
 Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe Thr Phe Gly Phe Glu  
 225 230 235 240  
 Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu Gly Ala Gly Lys Phe  
 245 250 255  
 Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu Leu Ile His Ala Gly  
 260 265 270

## Li et al., Degradable Clostridial Toxins

His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn	Arg	Val	Phe	Lys	Val
		275					280					285			
Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu	Glu	Val	Ser	Phe	Glu
		290				295					300				
Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys	Phe	Ile	Asp	Ser	Leu
305					310					315					320
Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn	Lys	Phe	Lys	Asp	Ile
				325					330					335	
Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val	Gly	Thr	Thr	Ala	Ser
			340					345					350		
Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys	Tyr	Leu	Leu	Ser	Glu
		355					360					365			
Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu	Lys	Phe	Asp	Lys	Leu
		370				375					380				
Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp	Phe	Val	Lys	Phe	
385					390					395					400
Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn	Phe	Asp	Lys	Ala	Val
				405					410					415	
Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr	Thr	Ile	Tyr	Asp	Gly
			420					425					430		
Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln	Asn
		435					440					445			
Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr	Gly
	450					455					460				
Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	Gly	Ile	Ile	Thr	Ser
465					470					475					480
Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Asn	Lys	Ala	Leu	Asn	Asp	Leu
				485					490					495	
Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp
			500					505					510		
Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr
		515					520					525			
Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln
		530				535					540				
Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile
545					550					555					560
Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn
				565					570					575	
Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr
			580					585					590		
Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg
		595					600					605			
Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg
		610				615					620				
Val	T														

## Li et al., Degradable Clostridial Toxins

				740				745					750			
Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	
		755					760					765				
Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	
	770					775					780					
Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	
785					790					795					800	
Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	
				805					810					815		
Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	
			820					825					830			
Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	
		835					840					845				
Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	
	850					855					860					
Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	
865					870					875					880	
Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	
				885					890					895		
Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	
			900					905					910			
Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	
		915					920					925				
Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	
	930					935					940					
Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	
945					950					955					960	
Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	
				965					970					975		
Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	
			980					985					990			
Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	
		995					1000					1005				
Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	
	1010					1015					1020					
Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	
1025					1030					1035					1040	
Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	
				1045					1050					1055		
Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	
			1060					1065					1070			
Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	
	</															

Li *et al.*, Degradable Clostridial Toxins

Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys  
 1220 1225 1230  
 Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile  
 1235 1240 1245  
 Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val  
 1250 1255 1260  
 Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met  
 1265 1270 1275 1280  
 Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His  
 1285 1290 1295  
 Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg  
 1300 1305 1310  
 Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile  
 1315 1320 1325  
 Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu  
 1330 1335

&lt;210&gt; 91

&lt;211&gt; 1356

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1356)

&lt;223&gt; BoNT/A-ED-PAR4-Thrombin

&lt;400&gt; 91

Met Trp Gly Arg Leu Leu Leu Trp Pro Leu Val Leu Gly Phe Ser Leu  
 1 5 10 15  
 Ser Gly Gly Thr Gln Thr Pro Ser Val Tyr Asp Glu Ser Gly Ser Thr  
 20 25 30  
 Gly Gly Gly Asp Asp Ser Thr Pro Ser Ile Leu Pro Ala Pro Arg Gly  
 35 40 45  
 Tyr Pro Gly Gln Val Cys Ala Asn Asp Ser Asp Thr Leu Pro Phe Val  
 50 55 60  
 Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly Val Asp Ile Ala  
 65 70 75 80  
 Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro Val Lys Ala Phe  
 85 90 95  
 Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg Asp Thr Phe Thr  
 100 105 110  
 Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu Ala Lys Gln Val  
 115 120 125  
 Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr Asp Asn Glu Lys  
 130 135 140  
 Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu Arg Ile Tyr Ser  
 145 150 155 160  
 Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val Arg Gly Ile Pro  
 165 170 175  
 Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys Val Ile Asp Thr  
 180 185 190  
 Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr Arg Ser Glu Glu  
 195 200 205  
 Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile Ile Gln Phe Glu  
 210 215 220  
 Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr Arg Asn Gly Tyr  
 225 230 235 240



## Li et al., Degradable Clostridial Toxins

Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe	Thr	Phe	Gly	Phe
				245					250					255	
Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu	Gly	Ala	Gly	Lys
			260					265					270		
Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu	Leu	Ile	His	Ala
			275					280					285		
Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn	Arg	Val	Phe	Lys
	290					295					300				
Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu	Glu	Val	Ser	Phe
305					310					315					320
Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys	Phe	Ile	Asp	Ser
				325					330					335	
Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn	Lys	Phe	Lys	Asp
			340					345					350		
Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val	Gly	Thr	Thr	Ala
			355				360					365			
Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys	Tyr	Leu	Leu	Ser
	370					375					380				
Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu	Lys	Phe	Asp	Lys
385					390					395					400
Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp	Asn	Phe	Val	Lys
				405					410					415	
Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn	Phe	Asp	Lys	Ala
			420					425					430		
Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr	Thr	Ile	Tyr	Asp
		435					440					445			
Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln
						455					460				
Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr
465					470					475					480
Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	Gly	Ile	Ile	Thr
				485					490					495	
Ser	Lys	Thr	Lys	Ser	Leu	Asp	Lys	Gly	Tyr	Asn	Lys	Ala	Leu	Asn	Asp
			500					505					510		
Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu
		515					520					525			
Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp
	530					535					540				
Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln
545					550					555					560
Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser
				565					570					575	
Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro
			580					585					590		
Asn															

## Li et al., Degradable Clostridial Toxins

705					710					715				720	
Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu
				725					730					735	
Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val
			740					745					750		
Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu
		755				760					765				
Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln
	770				775						780				
Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala
785				790						795					800
Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu
			805						810					815	
Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys
			820					825					830		
Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu
	835						840					845			
Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly
	850					855					860				
Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu
865				870						875					880
Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg
			885						890					895	
Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln
		900						905					910		
Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu
	915						920					925			
Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu
	930					935					940				
Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile
945				950						955					960
Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu
			965						970					975	
Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile
		980						985					990		
Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg
	995						1000						1005		
Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile
	1010					1015					1020				
Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr
1025				1030						1035					1040
Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg
			1045						1050					1055	
Val	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn
		1060						1065					1070		
Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys
	1075						1080					1085			
Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu
	1090					1095					1100				
Gly	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys
1105				1110						1115					1120
Arg	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp
			1125						1130					1135	
Lys	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser
		1140						1145					1150		
Asn	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp
	1155						1160					1165			

Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp

Li *et al.*, Degradable Clostridial Toxins

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      1170      1175      1180
Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg
1185      1190      1195      1200
Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg
      1205      1210      1215
Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn
      1220      1225      1230
Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn
      1235      1240      1245
Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys
      1250      1255      1260
Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val
1265      1270      1275      1280
Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys
      1285      1290      1295
Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe
      1300      1305      1310
His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn
      1315      1320      1325
Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe
      1330      1335      1340
Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
1345      1350      1355

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&lt;210&gt; 92

&lt;211&gt; 1348

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1348)

&lt;223&gt; BoNT/A-ED-PAR4-Xa

&lt;400&gt; 92

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Met Trp Gly Arg Leu Leu Leu Trp Pro Leu Val Leu Gly Phe Ser Leu
 1      5      10      15
Ser Gly Gly Thr Gln Thr Pro Ser Val Tyr Asp Glu Ser Gly Ser Thr
      20      25      30
Gly Gly Gly Asp Asp Ser Thr Pro Ser Ile Leu Ile Glu Gly Arg Gly
      35      40      45
Tyr Pro Gly Gln Val Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp
      50      55      60
Pro Val Asn Gly Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly
      65      70      75      80
Gln Met Gln Pro Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val
      85      90      95
Ile Pro Glu Arg Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn
      100      105      110
Pro Pro Pro Glu Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr
      115      120      125
Tyr Leu Ser Thr Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr
      130      135      140
Lys Leu Phe Glu Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu
145      150      155      160
Thr Ser Ile Val Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp
      165      170      175
Thr Glu Leu Lys Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro

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## Li et al., Degradable Clostridial Toxins

			180					185					190			
Asp	Gly	Ser	Tyr	Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	
		195					200					205				
Ser	Ala	Asp	Ile	Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	
	210					215					220					
Leu	Asn	Leu	Thr	Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	
225					230					235					240	
Ser	Pro	Asp	Phe	Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	
				245						250				255		
Asn	Pro	Leu	Leu	Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	
			260					265					270			
Leu	Ala	His	Glu	Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	
		275					280					285				
Ile	Asn	Pro	Asn	Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	
	290					295					300					
Met	Ser	Gly	Leu	Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	
305					310					315					320	
His	Asp	Ala	Lys	Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	
				325					330					335		
Tyr	Tyr	Tyr	Asn	Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	
			340					345					350			
Lys	Ser	Ile	Val	Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	
		355					360					365				
Phe	Lys	Glu	Lys	Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	
	370					375					380					
Val	Asp	Lys	Leu	Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	
385					390					395					400	
Tyr	Thr	Glu	Asp	Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	
				405					410					415		
Thr	Tyr	Leu	Asn	Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	
			420					425					430			
Lys	Val	Asn	Tyr	Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	
		435					440					445				
Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	
	450					455					460					
Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	
465					470					475					480	
Leu	Cys	Val	Arg	Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	
				485					490					495		
Gly	Arg	Asn	Lys	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	
			500					505					510			
Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	
		515					520					525				
Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	
	530					53										

## Li et al., Degradable Clostridial Toxins

Gly Trp Val Glu Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu  
 660 665 670  
 Val Ser Thr Thr Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr  
 675 680 685  
 Ile Gly Pro Ala Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe  
 690 695 700  
 Val Gly Ala Leu Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile  
 705 710 715 720  
 Pro Glu Ile Ala Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr  
 725 730 735  
 Ile Ala Asn Lys Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser  
 740 745 750  
 Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn  
 755 760 765  
 Trp Leu Ala Lys Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met  
 770 775 780  
 Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn  
 785 790 795 800  
 Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe  
 805 810 815  
 Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala  
 820 825 830  
 Met Ile Asn Ile Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu  
 835 840 845  
 Met Asn Ser Met Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp  
 850 855 860  
 Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly  
 865 870 875 880  
 Thr Leu Ile Gly Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr  
 885 890 895  
 Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln  
 900 905 910  
 Arg Leu Leu Ser Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr  
 915 920 925  
 Ser Ile Leu Asn Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser  
 930 935 940  
 Arg Tyr Ala Ser Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro  
 945 950 955 960  
 Ile Asp Lys Asn Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile  
 965 970 975  
 Glu Val Ile Leu Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn  
 980 985 990  
 Phe Ser Thr Ser Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile  
 995 1000 1005  
 Ser Leu Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser  
 1010 1015 1020  
 Gly Trp Lys Val Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln  
 1025 1030 1035 1040  
 Asp Thr Gln Glu Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met  
 1045 1050 1055  
 Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr  
 1060 1065 1070  
 Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile  
 1075 1080 1085  
 Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn  
 1090 1095 1100  
 Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp  
 1105 1110 1115 1120  
 Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile

## Li et al., Degradable Clostridial Toxins

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      1125      1130      1135
Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe
      1140      1145      1150
Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu
      1155      1160      1165
Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly
      1170      1175      1180
Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile
1185      1190      1195      1200
Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys
      1205      1210      1215
Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val
      1220      1225      1230
Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn
      1235      1240      1245
Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro
      1250      1255      1260
Asp Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp
1265      1270      1275      1280
Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly
      1285      1290      1295
Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys
      1300      1305      1310
Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg
      1315      1320      1325
Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly
      1330      1335      1340
Glu Arg Pro Leu
1345

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&lt;210&gt; 93

&lt;211&gt; 1306

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1306)

&lt;223&gt; BoNT/A-TD-PAR1-Thrombin

&lt;400&gt; 93

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1      5      10      15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
      20      25      30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
      35      40      45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
      50      55      60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
      65      70      75      80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
      85      90      95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
      100      105      110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
      115      120      125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr

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Li *et al.*, Degradable Clostridial Toxins

130	135	140
Arg Ser Glu Glu Leu Asn	Leu Val Ile Ile Gly	Pro Ser Ala Asp Ile
145	150	155
Ile Gln Phe Glu Cys Lys	Ser Phe Gly His Glu	Val Leu Asn Leu Thr
165	170	175
Arg Asn Gly Tyr Gly Ser	Thr Gln Tyr Ile Arg	Phe Ser Pro Asp Phe
180	185	190
Thr Phe Gly Phe Glu Glu	Ser Leu Glu Val Asp	Thr Asn Pro Leu Leu
195	200	205
Gly Ala Gly Lys Phe Ala	Thr Asp Pro Ala Val	Thr Leu Ala His Glu
210	215	220
Leu Ile His Ala Gly His	Arg Leu Tyr Gly Ile	Ala Ile Asn Pro Asn
225	230	235
Arg Val Phe Lys Val Asn	Thr Asn Ala Tyr Tyr	Glu Met Ser Gly Leu
245	250	255
Glu Val Ser Phe Glu Glu	Leu Arg Thr Phe Gly	Gly His Asp Ala Lys
260	265	270
Phe Ile Asp Ser Leu Gln	Glu Asn Glu Phe Arg	Leu Tyr Tyr Tyr Asn
275	280	285
Lys Phe Lys Asp Ile Ala	Ser Thr Leu Asn Lys	Ala Lys Ser Ile Val
290	295	300
Gly Thr Thr Ala Ser Leu	Gln Tyr Met Lys Asn	Val Phe Lys Glu Lys
305	310	315
Tyr Leu Leu Ser Glu Asp	Thr Ser Gly Lys Phe	Ser Val Asp Lys Leu
325	330	335
Lys Phe Asp Lys Leu Tyr	Lys Met Leu Thr Glu	Ile Tyr Thr Glu Asp
340	345	350
Asn Phe Val Lys Phe Phe	Lys Val Leu Asn Arg	Lys Thr Tyr Leu Asn
355	360	365
Phe Asp Lys Ala Val Phe	Lys Ile Asn Ile Val	Pro Lys Val Asn Tyr
370	375	380
Thr Ile Tyr Asp Gly Phe	Asn Leu Arg Asn Thr	Asn Leu Ala Ala Asn
385	390	395
Phe Asn Gly Gln Asn Thr	Glu Ile Asn Asn Met	Asn Phe Thr Lys Leu
405	410	415
Lys Asn Phe Thr Gly Leu	Phe Glu Phe Tyr Lys	Leu Leu Cys Val Arg
420	425	430
Gly Ile Ile Thr Ser Lys	Thr Lys Ser Leu Pro	Arg Ser Phe Leu Leu
435	440	445
Arg Asn Pro Asn Asp Lys	Tyr Glu Pro Phe Ala	Leu Asn Asp Leu Cys
450	455	460
Ile Lys Val Asn Asn Trp	Asp Leu Phe Phe Ser	Pro Ser Glu Asp Asn
465	470	475
Phe Thr Asn Asp Leu Asn	Lys Gly Glu Glu Ile	Thr Ser Asp Thr Asn
485	490	495
Ile Glu Ala Ala Glu Glu	Asn Ile Ser Leu Asp	Leu Ile Gln Gln Tyr
500	505	510
Tyr Leu Thr Phe Asn Phe	Asp Asn Glu Pro Glu	Asn Ile Ser Ile Glu
515	520	525
Asn Leu Ser Ser Asp Ile	Ile Ile Gly Gln Leu	Glu Leu Met Pro Asn Ile
530	535	540
Glu Arg Phe Pro Asn Gly	Lys Lys Tyr Glu Leu	Asp Lys Tyr Thr Met
545	550	555
Phe His Tyr Leu Arg Ala	Gln Glu Phe Glu His	Gly Lys Ser Arg Ile
565	570	575
Ala Leu Thr Asn Ser Val	Asn Glu Ala Leu Leu	Asn Pro Ser Arg Val
580	585	590
Tyr Thr Phe Ser Ser Asp	Tyr Val Lys Lys Val	Asn Lys Ala Thr
595	600	605

Li *et al.*, Degradable Clostridial Toxins

Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	610	615	620
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	625	630	635
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	645	650	655
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	660	665	670
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	675	680	685
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	690	695	700
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	705	710	715
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	725	730	735
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	740	745	750
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	755	760	765
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	770	775	780
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	785	790	795
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	805	810	815
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	820	825	830
Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	835	840	845
Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	850	855	860
Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	865	870	875
Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	885	890	895
His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	900	905	910
Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	915	920	925
Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	930	935	940
Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	945	950	955
Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	965	970	975
Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	980	985	990
Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	995	1000	1005
Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	1010	1015	1020
Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	1025	1030	1035
Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	1045	1050	1055
Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	1060	1065	1070



Li *et al.*, Degradable Clostridial Toxins

Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu  
 1075 1080 1085  
 Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser  
 1090 1095 1100  
 Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro  
 1105 1110 1115 1120  
 Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn  
 1125 1130 1135  
 Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser  
 1140 1145 1150  
 Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr  
 1155 1160 1165  
 Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val  
 1170 1175 1180  
 Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu  
 1185 1190 1195 1200  
 Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu  
 1205 1210 1215  
 Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val  
 1220 1225 1230  
 Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn  
 1235 1240 1245  
 Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln  
 1250 1255 1260  
 Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln  
 1265 1270 1275 1280  
 Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro  
 1285 1290 1295  
 Val Asp Asp Gly Trp Gly Glu Arg Pro Leu  
 1300 1305

&lt;210&gt; 94

&lt;211&gt; 1300

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1300)

&lt;223&gt; BoNT/A-TD-PAR1-Xa

&lt;400&gt; 94

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
 1 5 10 15  
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro  
 20 25 30  
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
 35 40 45  
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu  
 50 55 60  
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr  
 65 70 75 80  
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu  
 85 90 95  
 Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val  
 100 105 110  
 Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys

## Li et al., Degradable Clostridial Toxins

		115					120					125				
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr	
	130					135					140					
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile	
145					150					155					160	
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr	
				165					170					175		
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe	
			180					185					190			
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu	
		195					200					205				
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu	
	210					215					220					
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn	
225					230					235					240	
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu	
				245					250					255		
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys	
			260					265					270			
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn	
		275					280					285				
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val	
	290					295					300					
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys	
305					310					315					320	
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu	
				325					330					335		
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp	
			340					345					350			
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn	
		355					360					365				
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr	
	370					375					380					
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn	
385					390					395					400	
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	
				405					410					415		
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	
			420					425					430			
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Ser	Phe	
		435					440					445				
Leu	Leu	Arg	Asn	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	
		450				455					460					
Asp	Leu</															

## Li et al., Degradable Clostridial Toxins

										580						585						590						
Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu													
										595			600			605												
Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu													
										610			615			620												
Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr													
										625			630			635												
Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe													
										645			650			655												
Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile													
										660			665			670												
Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr													
										675			680			685												
Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser													
										690			695			700												
Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn													
										705			710			715												
Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met													
										725			730			735												
Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn													
										740			745			750												
Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe													
										755			760			765												
Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala													
										770			775			780												
Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu													
										785			790			795												
Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp													
										805			810			815												
Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly													
										820			825			830												
Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr													
										835			840			845												
Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln													
										850			855			860												
Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr													
										865			870			875												
Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser													
										885			890			895												
Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro													
										900			905			910												
Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile													
										915			920			925												
Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn													
										930			935			940												
Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile													
										945			950			955												
Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser													
										965			970			975												
Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln													
										980			985			990												
Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met													
										995			1000			1005												
Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr													
										1010			1015			1020												
Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile													
										1025			1030			1035												
Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His	Ala	Ser	Asn	Asn													

Li *et al.*, Degradable Clostridial Toxins

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                1045                1050                1055
Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp
                1060                1065                1070
Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile
                1075                1080                1085
Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe
                1090                1095                1100
Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu
1105                1110                1115                1120
Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly
                1125                1130                1135
Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile
                1140                1145                1150
Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys
                1155                1160                1165
Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val
                1170                1175                1180
Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn
1185                1190                1195                1200
Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro
                1205                1210                1215
Asp Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp
                1220                1225                1230
Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly
                1235                1240                1245
Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys
                1250                1255                1260
Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg
1265                1270                1275                1280
Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly
                1285                1290                1295
Glu Arg Pro Leu
                1300

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&lt;210&gt; 95

&lt;211&gt; 1306

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1306)

&lt;223&gt; BoNT/A-TD-PAR2-Trypsin

&lt;400&gt; 95

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1                5                10                15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
                20                25                30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
                35                40                45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
                50                55                60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65                70                75                80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
                85                90                95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val

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Li *et al.*, Degradable Clostridial Toxins

100						105						110					
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys		
115						120						125					
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr		
130						135						140					
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile		
145	150						155						160				
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr		
165						170						175					
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe		
180						185						190					
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu		
195						200						205					
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu		
210						215						220					
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn		
225	230						235						240				
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu		
245						250						255					
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys		
260						265						270					
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn		
275						280						285					
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val		
290						295						300					
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys		
305	310						315						320				
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu		
325						330						335					
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp		
340						345						350					
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn		
355						360						365					
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr		
370						375						380					
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn		
385	390						395						400				
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu		
405						410						415					
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg		
420						425						430					
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Gly	Arg	Ser	Leu	Ile	Gly		
435						440						445					
Lys	Val	Asp	Gly	Thr	Ser	His	Val	Thr	Gly	Ala	Leu	Asn	Asp	Leu	Cys		
450						455						460					
Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn		
465	470						475						480				
Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn		
485						490						495					
Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr		
500						505						510					
Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu		
515						520						525					
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile		
530						535						540					
Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met		
545	550						555						560				
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile		
565						570						575					

Li *et al.*, Degradable Clostridial Toxins

Ala Leu Thr Asn Ser Val Asn Glu Ala Leu Leu Asn Pro Ser Arg Val  
 580 585 590  
 Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys Lys Val Asn Lys Ala Thr  
 595 600 605  
 Glu Ala Ala Met Phe Leu Gly Trp Val Glu Gln Leu Val Tyr Asp Phe  
 610 615 620  
 Thr Asp Glu Thr Ser Glu Val Ser Thr Thr Asp Lys Ile Ala Asp Ile  
 625 630 635 640  
 Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Met  
 645 650 655  
 Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu Ile Phe Ser Gly Ala Val  
 660 665 670  
 Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala Ile Pro Val Leu Gly Thr  
 675 680 685  
 Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys Val Leu Thr Val Gln Thr  
 690 695 700  
 Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr  
 705 710 715 720  
 Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys Val Asn Thr Gln Ile Asp  
 725 730 735  
 Leu Ile Arg Lys Lys Met Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala  
 740 745 750  
  
 Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu  
 755 760 765  
 Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn  
 770 775 780  
 Glu Ser Ile Asn Lys Ala Met Ile Asn Ile Asn Lys Phe Leu Asn Gln  
 785 790 795 800  
 Cys Ser Val Ser Tyr Leu Met Asn Ser Met Ile Pro Tyr Gly Val Lys  
 805 810 815  
 Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr  
 820 825 830  
 Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys  
 835 840 845  
 Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser  
 850 855 860  
 Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr Phe Thr Glu Tyr Ile  
 865 870 875 880  
 Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn Leu Arg Tyr Glu Ser Asn  
 885 890 895  
 His Leu Ile Asp Leu Ser Arg Tyr Ala Ser Lys Ile Asn Ile Gly Ser  
 900 905 910  
 Lys Val Asn Phe Asp Pro Ile Asp Lys Asn Gln Ile Gln Leu Phe Asn  
 915 920 925  
 Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn Ala Ile Val Tyr  
 930 935 940  
 Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp Ile Arg Ile Pro  
 945 950 955 960  
 Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn Glu Tyr Thr Ile Ile Asn  
 965 970 975  
 Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu Asn Tyr Gly Glu  
 980 985 990  
 Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu Ile Lys Gln Arg Val Val  
 995 1000 1005  
 Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp  
 1010 1015 1020  
 Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr  
 1025 1030 1035 1040

## Li et al.; Degradable Clostridial Toxins

Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn  
 1045 1050 1055  
 Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg Asp  
 1060 1065 1070  
 Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu  
 1075 1080 1085  
 Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser  
 1090 1095 1100  
 Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro  
 1105 1110 1115 1120  
 Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn  
 1125 1130 1135  
 Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser  
 1140 1145 1150  
 Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr  
 1155 1160 1165  
 Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val  
 1170 1175 1180  
 Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu  
 1185 1190 1195 1200  
 Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu  
 1205 1210 1215  
 Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val  
 1220 1225 1230  
 Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn  
 1235 1240 1245  
 Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln  
 1250 1255 1260  
 Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln  
 1265 1270 1275 1280  
 Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro  
 1285 1290 1295  
 Val Asp Asp Gly Trp Gly Glu Arg Pro Leu  
 1300 1305

&lt;210&gt; 96

&lt;211&gt; 1300

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1300)

&lt;223&gt; BoNT/A-TD-PAR2-Xa

&lt;400&gt; 96

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
 1 5 10 15  
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro  
 20 25 30  
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
 35 40 45  
 Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu  
 50 55 60  
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr  
 65 70 75 80  
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu

## Li et al., Degradable Clostridial Toxins

				85					90				95				
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val		
			100					105					110				
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys		
		115					120					125					
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr		
		130				135					140						
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile		
145					150					155					160		
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr		
				165				170						175			
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe		
		180						185					190				
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu		
		195					200					205					
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu		
	210					215					220						
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn		
225					230					235					240		
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu		
				245				250						255			
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys		
		260						265					270				
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn		
	275						280					285					
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val		
	290					295				300							
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys		
305					310					315					320		
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu		
			325					330						335			
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp		
		340					345					350					
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn		
	355					360						365					
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr		
	370					375				380							
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn		
385					390					395					400		
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu		
			405					410						415			
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg		
		420					425					430					
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Ser	Leu		
	435						440					445					
Ile	Gly	Lys	Val	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp		
	450					455				460							
Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn		
465					470					475					480		
Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu		
			485					490						495			
Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe		
		500						505					510				
Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile		
	515						520					525					
Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly		
	530					535				540							
Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala		



## Li et al., Degradable Clostridial Toxins

545					550					555				560
Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser
				565					570					575
Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser
			580						585				590	
Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe
		595					600					605		Leu
Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser
	610						615				620			Glu
Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro
625					630					635				640
Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp
				645				650					655	Phe
Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe
		660					665					670		Ile
Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser
	675						680				685			Tyr
Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu
	690					695				700				Ser
Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr
705					710					715				Asn
Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys
			725						730					Met
Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Asn
		740					745					750		
Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn
	755					760					765			Phe
Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys
	770					775				780				Ala
Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr
785					790				795					Leu
Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe
			805					810						Asp
Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg
		820					825					830		Gly
Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn
	835					840						845		Thr
Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn
	850					855				860				Gln
Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn
865					870				875					Thr
Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu
			885					890					895	Ser
Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp
	900						905					910		Pro
Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys
	915					920					925			Ile
Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu
	930					935				940				Asn
Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser
945					950				955					Ile
Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn
			965					970					975	Ser
Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu
		980					985					990		Gln
Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln
	995					1000						1005		Met
Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile

## Li et al., Degradable Clostridial Toxins

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1010      1015      1020
Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile
1025      1030      1035      1040
Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn
1045      1050      1055
Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp
1060      1065      1070
Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile
1075      1080      1085
Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe
1090      1095      1100
Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu
1105      1110      1115      1120
Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly
1125      1130      1135
Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile
1140      1145      1150
Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys
1155      1160      1165
Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val
1170      1175      1180
Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn
1185      1190      1195      1200
Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro
1205      1210      1215
Asp Val Gly Asn Leu Ser Gln Val Val Met Lys Ser Lys Asn Asp
1220      1225      1230
Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly
1235      1240      1245
Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys
1250      1255      1260
Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg
1265      1270      1275      1280
Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly
1285      1290      1295
Glu Arg Pro Leu
1300

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&lt;210&gt; 97

&lt;211&gt; 1306

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1306)

&lt;223&gt; BoNT/A-TD-PAR3-Thrombin

&lt;400&gt; 97

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1      5      10      15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20      25      30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35      40      45
Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu
50      55      60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr

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Li *et al.*, Degradable Clostridial Toxins

65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115					120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165				170						175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
			245					250						255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
	275						280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295				300					
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
			325					330						335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
			405					410						415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Lys	Thr	Phe	Arg	Gly
		435					440					445			
Ala	Pro	Pro	Asn	Ser	Phe	Glu	Glu	Phe	Pro	Ala	Leu	Asn	Asp	Leu	Cys
	450					455					460				
Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn
465					470					475					480
Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn
			485					490						495	
Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr
		500					505					510			
Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu
	515						520					525			
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile

Li *et al.*, Degradable Clostridial Toxins

530						535					540				
Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met
545					550					555					560
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile
				565					570						575
Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val
			580					585					590		
Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr
	595					600						605			
Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe
610					615						620				
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile
625					630					635					640
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met
				645					650					655	
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val
			660					665					670		
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr
	675						680					685			
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr
690						695					700				
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr
705					710					715					720
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp
				725					730					735	
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala
			740					745					750		
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu
	755						760					765			
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn
	770					775					780				
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln
785					790					795					800
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys
				805					810					815	
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr
			820					825					830		
Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys
	835					840						845			
Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser
	850					855					860				
Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile
865					870					875					880
Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn
				885					890					895	
His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser
			900					905					910		
Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn
			915					920					925		
Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr
	930					935						940			
Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro
945					950					955					960
Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn
				965					970					975	
Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu
			980					985					990		
Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val
	995						1000					1005			

Li *et al.*, Degradable Clostridial Toxins

Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp  
 1010 1015 1020  
 Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr  
 1025 1030 1035 1040  
 Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn  
 1045 1050 1055  
 Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg Asp  
 1060 1065 1070  
 Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu  
 1075 1080 1085  
 Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser  
 1090 1095 1100  
 Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro  
 1105 1110 1115 1120  
 Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn  
 1125 1130 1135  
 Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser  
 1140 1145 1150  
 Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr  
 1155 1160 1165  
 Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val  
 1170 1175 1180  
 Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu  
 1185 1190 1195 1200  
 Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu  
 1205 1210 1215  
 Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val  
 1220 1225 1230  
 Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn  
 1235 1240 1245  
 Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln  
 1250 1255 1260  
 Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln  
 1265 1270 1275 1280  
 Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro  
 1285 1290 1295  
 Val Asp Asp Gly Trp Gly Glu Arg Pro Leu  
 1300 1305

&lt;210&gt; 98

&lt;211&gt; 1300

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1300)

&lt;223&gt; BoNT/A-TD-PAR3-Xa

&lt;400&gt; 98

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
 1 5 10 15  
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro  
 20 25 30  
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
 35 40 45  
 Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu  
 50 55 60

Li *et al.*, Degradable Clostridial Toxins

Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115					120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165				170						175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
				245				250						255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
		275					280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
				325				330						335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
				405				410						415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420				425						430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Thr	Phe
		435					440					445			
Arg	Gly	Ala	Pro	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp
	450					455					460				
Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn
465					470					475					480
Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu
				485				490						495	
Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe
		500						505					510		
Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile
		515					520					525			
Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly

Li *et al.*, Degradable Clostridial Toxins

530		535		540
Lys Lys Tyr Glu Leu Asp	Lys Tyr Thr Met Phe His Tyr Leu Arg Ala			
545	550	555	560	
Gln Glu Phe Glu His Gly	Lys Ser Arg Ile Ala Leu Thr Asn Ser Val			
	565	570	575	
Asn Glu Ala Leu Leu Asn	Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser			
	580	585	590	
Asp Tyr Val Lys Lys Val	Asn Lys Ala Thr Glu Ala Ala Met Phe Leu			
	595	600	605	
Gly Trp Val Glu Gln Leu	Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu			
	610	615	620	
Val Ser Thr Thr Asp Lys	Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr			
625	630	635	640	
Ile Gly Pro Ala Leu Asn	Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe			
	645	650	655	
Val Gly Ala Leu Ile Phe	Ser Gly Ala Val Ile Leu Leu Glu Phe Ile			
	660	665	670	
Pro Glu Ile Ala Ile Pro	Val Leu Gly Thr Phe Ala Leu Val Ser Tyr			
	675	680	685	
Ile Ala Asn Lys Val Leu	Thr Val Gln Thr Ile Asp Asn Ala Leu Ser			
	690	695	700	
Lys Arg Asn Glu Lys Trp	Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn			
705	710	715	720	
Trp Leu Ala Lys Val Asn	Thr Gln Ile Asp Leu Ile Arg Lys Lys Met			
	725	730	735	
Lys Glu Ala Leu Glu Asn	Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn			
	740	745	750	
Tyr Gln Tyr Asn Gln Tyr	Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe			
	755	760	765	
Asn Ile Asp Asp Leu Ser	Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala			
	770	775	780	
Met Ile Asn Ile Asn Lys	Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu			
785	790	795	800	
Met Asn Ser Met Ile Pro	Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp			
	805	810	815	
Ala Ser Leu Lys Asp Ala	Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly			
	820	825	830	
Thr Leu Ile Gly Gln Val	Asp Arg Leu Lys Asp Lys Val Asn Asn Thr			
	835	840	845	
Leu Ser Thr Asp Ile Pro	Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln			
	850	855	860	
Arg Leu Leu Ser Thr Phe	Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr			
865	870	875	880	
Ser Ile Leu Asn Leu Arg	Tyr Glu Ser Asn His Leu Ile Asp Leu Ser			
	885	890	895	
Arg Tyr Ala Ser Lys Ile	Asn Ile Gly Ser Lys Val Asn Phe Asp Pro			
	900	905	910	
Ile Asp Lys Asn Gln Ile	Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile			
	915	920	925	
Glu Val Ile Leu Lys Asn	Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn			
	930	935	940	
Phe Ser Thr Ser Phe Trp	Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile			
945	950	955	960	
Ser Leu Asn Asn Glu Tyr	Thr Ile Ile Asn Cys Met Glu Asn Asn Ser			
	965	970	975	
Gly Trp Lys Val Ser Leu	Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln			
	980	985	990	
Asp Thr Gln Glu Ile Lys	Gln Arg Val Val Phe Lys Tyr Ser Gln Met			
	995	1000	1005	

## Li et al., Degradable Clostridial Toxins

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Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr
  1010                      1015                      1020
Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile
1025                      1030                      1035                      1040
Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn
                      1045                      1050                      1055
Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp
                      1060                      1065                      1070
Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile
                      1075                      1080                      1085
Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe
                      1090                      1095                      1100
Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu
1105                      1110                      1115                      1120
Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly
                      1125                      1130                      1135
Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile
                      1140                      1145                      1150
Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys
                      1155                      1160                      1165
Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val
                      1170                      1175                      1180
Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn
1185                      1190                      1195                      1200
Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro
                      1205                      1210                      1215
Asp Val Gly Asn Leu Ser Gln Val Val Met Lys Ser Lys Asn Asp
                      1220                      1225                      1230
Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly
                      1235                      1240                      1245
Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys
                      1250                      1255                      1260
Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg
1265                      1270                      1275                      1280
Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly
                      1285                      1290                      1295
Glu Arg Pro Leu
                      1300

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&lt;210&gt; 99

&lt;211&gt; 1306

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1306)

&lt;223&gt; BoNT/A-TD-PAR4-Thrombin

&lt;400&gt; 99

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
  1                      5                      10                      15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
                      20                      25                      30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
                      35                      40                      45
Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu
                      50                      55                      60

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## Li et al., Degradable Clostridial Toxins

Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115					120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165					170					175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
			245					250						255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
		275					280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
				325				330						335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
				405				410						415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Pro	Arg	Gly	Tyr	Pro	Gly
		435					440					445			
Gln	Val	Cys	Ala	Asn	Asp	Ser	Asp	Thr	Leu	Ala	Leu	Asn	Asp	Leu	Cys
	450					455					460				
Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn
465					470					475					480
Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn
				485				490						495	
Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr
		500					505						510		
Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu
		515					520					525			
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile

Li *et al.*, Degradable Clostridial Toxins

530						535						540					
Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met		
545					550					555					560		
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile		
				565					570						575		
Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val		
			580					585						590			
Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr		
		595				600						605					
Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe		
610					615						620						
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile		
625					630					635					640		
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met		
				645					650					655			
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val		
			660					665					670				
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr		
		675					680					685					
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr		
690					695						700						
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr		
705					710					715					720		
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp		
			725						730					735			
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala		
		740						745					750				
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu		
		755					760					765					
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn		
770					775						780						
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln		
785					790					795					800		
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys		
				805					810					815			
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr		
			820					825					830				
Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys		
		835					840					845					
Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser		
850					855						860						
Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile		
865					870					875					880		
Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn		
				885					890					895			
His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser		
		900						905					910				
Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn		
		915					920					925					
Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr		
930					935						940						
Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro		
945					950					955					960		
Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn		
			965						970					975			
Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu		
		980							985					990			
Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val		
995							1000					1005					

## Li et al., Degradable Clostridial Toxins

Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp  
 1010 1015 1020  
 Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr  
 1025 1030 1035 1040  
 Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn  
 1045 1050 1055  
 Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg Asp  
 1060 1065 1070  
 Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu  
 1075 1080 1085  
 Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser  
 1090 1095 1100  
 Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro  
 1105 1110 1115 1120  
 Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn  
 1125 1130 1135  
 Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser  
 1140 1145 1150  
 Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr  
 1155 1160 1165  
 Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val  
 1170 1175 1180  
 Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu  
 1185 1190 1195 1200  
 Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu  
 1205 1210 1215  
 Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val  
 1220 1225 1230  
 Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn  
 1235 1240 1245  
 Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln  
 1250 1255 1260  
 Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln  
 1265 1270 1275 1280  
 Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro  
 1285 1290 1295  
 Val Asp Asp Gly Trp Gly Glu Arg Pro Leu  
 1300 1305

&lt;210&gt; 100

&lt;211&gt; 1300

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1300)

&lt;223&gt; BoNT/A-TD-PAR4-Xa

&lt;400&gt; 100

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
 1 5 10 15  
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro  
 20 25 30  
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
 35 40 45  
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu

## Li et al., Degradable Clostridial Toxins

50	55	60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr		
65	70	75
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu		80
	85	90
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val		95
	100	105
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys		110
	115	120
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr		125
	130	135
Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile		140
145	150	155
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr		160
	165	170
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe		175
	180	185
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu		190
	195	200
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu		205
	210	215
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn		220
225	230	235
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu		240
	245	250
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys		255
	260	265
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn		270
	275	280
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val		285
	290	295
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys		300
305	310	315
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu		320
	325	330
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp		335
	340	345
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn		350
	355	360
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr		365
	370	375
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn		380
385	390	395
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu		400
	405	410
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg		415
	420	425
Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Ile Glu Gly Arg Gly Tyr		430
	435	440
Pro Gly Gln Val Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp		445
	450	455
Asp Leu Phe Phe Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn		460
465	470	475
Lys Gly Glu Glu Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu		480
	485	490
Asn Ile Ser Leu Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe		495
	500	505
Asp Asn Glu Pro Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile		510
	515	520
		525

## Li et al., Degradable Clostridial Toxins

Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly	530	535	540
Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala	545	550	555
Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val	565	570	575
Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser	580	585	590
Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu	595	600	605
Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu	610	615	620
Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr	625	630	635
Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe	645	650	655
Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile	660	665	670
Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr	675	680	685
Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser	690	695	700
Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn	705	710	715
Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met	725	730	735
Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn	740	745	750
Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	755	760	765
Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	770	775	780
Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	785	790	795
Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	805	810	815
Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	820	825	830
Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr	835	840	845
Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln	850	855	860
Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	865	870	875
Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	885	890	895
Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	900	905	910
Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	915	920	925
Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	930	935	940
Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	945	950	955
Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	965	970	975
Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	980	985	990
Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met			

## Li et al., Degradable Clostridial Toxins

995	1000	1005
Ile Asn Ile Ser Asp Tyr	Ile Asn Arg Trp Ile	Phe Val Thr Ile Thr
1010	1015	1020
Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr	Ile Asn Gly Arg Leu Ile	
1025	1030	1035
Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn		1040
1045	1050	1055
Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp		
1060	1065	1070
Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile		
1075	1080	1085
Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe		
1090	1095	1100
Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu		
1105	1110	1115
Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly		
1125	1130	1135
Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile		
1140	1145	1150
Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys		
1155	1160	1165
Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val		
1170	1175	1180
Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn		
1185	1190	1195
Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro		
1205	1210	1215
Asp Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp		
1220	1225	1230
Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly		
1235	1240	1245
Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys		
1250	1255	1260
Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg		
1265	1270	1275
Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly		
1285	1290	1295
Glu Arg Pro Leu		
1300		

&lt;210&gt; 101

&lt;211&gt; 1329

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1329)

&lt;223&gt; BoNT/A-BD-PAR1-Thrombin

&lt;400&gt; 101

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1 5 10 15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20 25 30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg

35

40

45

## Li et al., Degradable Clostridial Toxins

Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro	Glu
50						55				60					
Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
	115					120						125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165					170					175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
			195				200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
				245					250					255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
			275				280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
			290				295				300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
				325					330					335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
			355				360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
				405					410					415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Pro	Arg	Ser	Phe	Leu	Leu
			435				440					445			
Arg	Asn	Pro	Asn	Asp	Lys	Tyr	Glu	Pro	Phe	Ala	Leu	Asn	Asp	Leu	Phe
						455				460					
Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg
465					470					475					480
Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile
				485					490					495	
Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile
				500				505					510		
Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn

Li *et al.*, Degradable Clostridial Toxins

515					520					525					
Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp
530					535					540					
Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr
545					550					555					
Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu
565					570					575					
Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys
580					585					590					
Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr
595					600					605					
Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn
610					615					620					
Ser	Lys	Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser
625					630					635					
Asn	Leu	Gly	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp
645					650					655					
Gly	Cys	Arg	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu
660					665					670					
Phe	Asp	Lys	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn
675					680					685					
Gln	Ser	Asn	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln
690					695					700					
Tyr	Asp	Lys	Pro	Tyr	Tyr	Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr
705					710					715					
Val	Asp	Val	Asn	Asn	Val	Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly
725					730					735					
Pro	Arg	Gly	Ser	Val	Met	Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu
740					745					750					
Tyr	Arg	Gly	Thr	Lys	Phe	Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys
755					760					765					
Asp	Asn	Ile	Val	Arg	Asn	Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val
770					775					780					
Lys	Asn	Lys	Glu	Tyr	Arg	Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val
785					790					795					
Glu	Lys	Ile	Leu	Ser	Ala	Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser
805					810					815					
Gln	Val	Val	Val	Met	Lys	Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys
820					825					830					
Cys	Lys	Met	Asn	Leu	Gln	Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile
835					840					845					
Gly	Phe	His	Gln	Phe	Asn	Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp
850					855					860					
Tyr	Asn	Arg	Gln	Ile	Glu	Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp
865					870					875					
Glu	Phe	Ile	Pro	Val	Asp	Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu
885					890					895					
Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
900					905					910					
Ala	Leu	Val	Leu	Gln	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe
915					920					925					
Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu
930					935					940					
Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu
945					950					955					
Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro
965					970					975					
Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu
980					985					990					



Li *et al.*, Degradable Clostridial Toxins

Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu  
           995                          1000                          1005  
 Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu  
           1010                          1015                          1020  
 His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu  
           1025                          1030                          1035                          1040  
 Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys  
                           1045                          1050                          1055  
 Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu  
                           1060                          1065                          1070  
 Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr  
           1075                          1080                          1085  
 Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala  
           1090                          1095                          1100  
 Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu  
           1105                          1110                          1115                          1120  
 Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala  
                           1125                          1130                          1135  
 Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys  
                           1140                          1145                          1150  
 Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu  
           1155                          1160                          1165  
 Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys  
           1170                          1175                          1180  
 Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu  
           1185                          1190                          1195                          1200  
 Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn  
                           1205                          1210                          1215  
 Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp  
           1220                          1225                          1230  
 Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile  
           1235                          1240                          1245  
 Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met  
           1250                          1255                          1260  
 Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys  
           1265                          1270                          1275                          1280  
 Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly  
                           1285                          1290                          1295  
 Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp  
           1300                          1305                          1310  
 Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser  
           1315                          1320                          1325  
 Thr

<210> 102  
 <211> 1323  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> PEPTIDE  
 <222> (1)...(1323)  
 <223> BoNT/A-BD-PAR1-Xa

<400> 102  
 Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
   1                          5                          10                          15

## Li et al., Degradable Clostridial Toxins

Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn	Ala	Gly	Gln	Met	Gln	Pro
			20					25					30		
Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile	Trp	Val	Ile	Pro	Glu	Arg
		35					40					45			
Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro	Glu
	50					55					60				
Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70				75						80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115					120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165					170					175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
				245					250					255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
		275					280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
		290					295				300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
				325					330					335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe										

## Li et al., Degradable Clostridial Toxins

485										490				495			
Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu		
			500				505						510				
Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser		
			515				520						525				
Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr		
			530				535						540				
Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met		
			545				550						555				
Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile		
			565				570						575				
Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys		
			580				585						590				
Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe		
			595				600						605				
Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn		
			610				615						620				
Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His		
			625				630						635				
Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr	His		
			645				650						655				
Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu	Asn		
			660				665						670				
Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly	Ile		
			675				680						685				
Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr	Tyr		
			690				695						700				
Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn	Val		
			705				710						715				
Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val	Met		
			725				730						735				
Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys	Phe		
			740				745						750				
Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg	Asn		
			755				760						765				
Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr	Arg		
			770				775						780				
Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val	Glu	Lys	Ile	Leu	Ser	Ala		
			785				790						795				
Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser	Gln	Val	Val	Val	Met	Lys		
			805				810						815				
Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys	Cys	Lys	Met	Asn	Leu	Gln		
			820				825						830				
Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile	Gly	Phe	His	Gln	Phe	Asn		
			835				840						845				
Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp	Tyr	Asn	Arg	Gln	Ile	Glu		
			850				855						860				
Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp	Glu	Phe	Ile	Pro	Val	Asp		
			865				870						875				
Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu	Ala	Gly	Gly	Gly	Gly	Ser		
			885				890						895				
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Leu	Val	Leu	Gln	Cys		
			900				905						910				
Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn		
			915				920						925				
Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn		
			930				935						940				
Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr		
			945				950						955				
														960			

## Li et al., Degradable Clostridial Toxins

Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu
				965					970					975	
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile
			980					985					990		
Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met
		995					1000					1005			
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile
	1010					1015					1020				
Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val
1025					1030					1035					1040
Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr
				1045					1050					1055	
Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe
			1060					1065					1070		
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile
		1075					1080					1085			
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met
	1090					1095					1100				
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val
1105					1110					1115					1120
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr
				1125					1130					1135	
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr
			1140					1145					1150		
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr
		1155					1160					1165			
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp
	1170					1175					1180				
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala
1185					1190					1195					1200
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu
				1205					1210					1215	
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn
			1220					1225					1230		
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln
		1235					1240					1245			
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys
	1250					1255					1260				
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr
1265					1270					1275					1280
Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys
				1285											

<210> 103

<211> 1329

<212> PRT

<213> Artificial Sequence

**<220>**

<221> PEPTIDE

<222> (1) ... (1329)

<223> BoNT/A-BD-PAR2-Trypsin

<400> 103

## Li et al., Degradable Clostridial Toxins

Met	Pro	Phe	Val	Asn	Lys	Gln	Phe	Asn	Tyr	Lys	Asp	Pro	Val	Asn	Gly
1				5					10					15	
Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn	Ala	Gly	Gln	Met	Gln	Pro
			20					25					30		
Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile	Trp	Val	Ile	Pro	Glu	Arg
		35					40					45			
Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro	Glu
	50					55					60				
Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115					120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165					170					175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
		180						185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
				245					250					255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
		275					280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
				325					330					335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
				405					410					415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Gly	Arg	Ser	Leu	Ile	Gly
	435						440					445			
Lys	Val	Asp	Gly	Thr	Ser	His	Val	Thr	Gly	Ala	Leu	Asn	Asp	Leu	Phe
	450					455					460				
Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg

## Li et al., Degradable Clostridial Toxins

465					470					475					480
Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile
				485					490					495	
Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile
			500					505					510		
Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn
		515					520					525			
Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp
	530					535				540					
Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr
545					550					555				560	
Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu
			565					570					575		
Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys
		580					585					590			
Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr
	595					600				605					
Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn
610					615					620					
Ser	Lys	Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser
625				630					635					640	
Asn	Leu	Gly	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp
			645					650					655		
Gly	Cys	Arg	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu
		660					665					670			
Phe	Asp	Lys	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn
	675					680					685				
Gln	Ser	Asn	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln
	690				695					700					
Tyr	Asp	Lys	Pro	Tyr	Tyr	Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr
705				710					715					720	
Val	Asp	Val	Asn	Asn	Val	Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly
			725					730					735		
Pro	Arg	Gly	Ser	Val	Met	Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu
		740					745					750			
Tyr	Arg	Gly	Thr	Lys	Phe	Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys
	755					760				765					
Asp	Asn	Ile	Val	Arg	Asn	Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val
	770				775					780					
Lys	Asn	Lys	Glu	Tyr	Arg	Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val
785				790					795					800	
Glu	Lys	Ile	Leu	Ser	Ala	Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser
			805					810					815		
Gln	Val	Val	Val	Met	Lys	Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys
		820					825					830			
Cys	Lys	Met	Asn	Leu	Gln	Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile
	835					840				845					
Gly	Phe	His	Gln	Phe	Asn	Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp
	850				855					860					
Tyr	Asn	Arg	Gln	Ile	Glu	Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp
865				870					875					880	
Glu	Phe	Ile	Pro	Val	Asp	Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu
			885				890						895		
Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
		900					905					910			
Ala	Leu	Val	Leu	Gln	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe
	915					920					925				
Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu
930					935					940					

## Li et al., Degradable Clostridial Toxins

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu  
 945 950 955 960  
 Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro  
 965 970 975  
 Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu  
 980 985 990  
 Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu  
 995 1000 1005  
 Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu  
 1010 1015 1020  
 His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu  
 1025 1030 1035 1040  
 Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys  
 1045 1050 1055  
 Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu  
 1060 1065 1070  
 Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr  
 1075 1080 1085  
 Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala  
 1090 1095 1100  
 Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu  
 1105 1110 1115 1120  
 Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala  
 1125 1130 1135  
 Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys  
 1140 1145 1150  
 Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu  
 1155 1160 1165  
 Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys  
 1170 1175 1180  
 Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu  
 1185 1190 1195 1200  
 Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn  
 1205 1210 1215  
 Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp  
 1220 1225 1230  
 Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile  
 1235 1240 1245  
 Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met  
 1250 1255 1260  
 Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys  
 1265 1270 1275 1280  
 Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly  
 1285 1290 1295  
 Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp  
 1300 1305 1310  
 Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser  
 1315 1320 1325  
 Thr

&lt;210&gt; 104

&lt;211&gt; 1323

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

## Li et al., Degradable Clostridial Toxins

&lt;222&gt; (1) ... (1323)

&lt;223&gt; BoNT/A-BD-PAR2-Xa

&lt;400&gt; 104

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1          5          10          15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
 20          25          30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35          40          45
Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu
 50          55          60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
 65          70          75          80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
 85          90          95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
 100          105          110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
 115          120          125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
 130          135          140
Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
 145          150          155          160
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
 165          170          175
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
 180          185          190
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
 195          200          205
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
 210          215          220
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
 225          230          235          240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
 245          250          255
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
 260          265          270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
 275          280          285
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
 290          295          300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
 305          310          315          320
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
 325          330          335
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
 340          345          350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
 355          360          365
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
 370          375          380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
 385          390          395          400
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
 405          410          415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
 420          425          430
Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Ile Glu Gly Arg Ser Leu

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Li *et al.*, Degradable Clostridial Toxins

		435					440					445			
Ile	Gly	Lys	Val	Ala	Leu	Asn	Asp	Leu	Phe	Thr	Glu	Tyr	Ile	Lys	Asn
	450					455					460				
Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu
465					470					475					480
Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val
				485					490					495	
Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu
		500					505					510			
Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser
	515					520						525			
Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr
	530					535					540				
Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met
545					550				555						560
Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile
				565				570						575	
Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys
		580					585					590			
Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe
	595					600					605				
Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn
	610					615					620				
Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His
625					630				635						640
Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr	His
				645					650					655	
Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu	Asn
		660						665					670		
Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly	Ile
		675					680					685			
Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr	Tyr
	690					695					700				
Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn	Val
705					710						715				720
Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val	Met
				725					730					735	
Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys	Phe
		740						745					750		
Ile	Ile	Lys	Tyr	Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg	Asn	
		755					760					765			
Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr	Arg
	770					775					780				
Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val	Glu	Lys	Ile	Leu	Ser	Ala
785					790					795					800
Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser	Gln	Val	Val	Val	Met	Lys
				805					810					815	
Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys	Cys	Lys	Met	Asn	Leu	Gln
				820				825					830		
Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile	Gly	Phe	His	Gln	Phe	Asn
		835					840					845			
Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp	Tyr	Asn	Arg	Gln	Ile	Glu
	850					855					860				
Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp	Glu	Phe	Ile	Pro	Val	Asp
865					870					875					880
Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu	Ala	Gly	Gly	Gly	Gly	Ser
				885					890					895	
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Leu	Val	Leu	Gln	Cys
		900					905						910		

## Li et al., Degradable Clostridial Toxins

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Ile Lys Val Asn Asn Trp Asp Leu Phe Phe Ser Pro Ser Glu Asp Asn
   915                               920               925
Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu Ile Thr Ser Asp Thr Asn
   930                               935               940
Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu Asp Leu Ile Gln Gln Tyr
  945                               950               955               960
Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro Glu Asn Ile Ser Ile Glu
                965                               970               975
Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu Glu Leu Met Pro Asn Ile
                980                               985               990
Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu Leu Asp Lys Tyr Thr Met
   995                               1000               1005
Phe His Tyr Leu Arg Ala Gln Glu Phe Glu His Gly Lys Ser Arg Ile
  1010                               1015               1020
Ala Leu Thr Asn Ser Val Asn Glu Ala Leu Leu Asn Pro Ser Arg Val
 1025                               1030               1035               1040
Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys Lys Val Asn Lys Ala Thr
                1045                               1050               1055
Glu Ala Ala Met Phe Leu Gly Trp Val Glu Gln Leu Val Tyr Asp Phe
                1060                               1065               1070
Thr Asp Glu Thr Ser Glu Val Ser Thr Thr Asp Lys Ile Ala Asp Ile
                1075                               1080               1085
Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Met
 1090                               1095               1100
Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu Ile Phe Ser Gly Ala Val
 1105                               1110               1115               1120
Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala Ile Pro Val Leu Gly Thr
                1125                               1130               1135
Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys Val Leu Thr Val Gln Thr
                1140                               1145               1150
Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr
                1155                               1160               1165
Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys Val Asn Thr Gln Ile Asp
 1170                               1175               1180
Leu Ile Arg Lys Lys Met Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala
 1185                               1190               1195               1200
Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu
                1205                               1210               1215
Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn
                1220                               1225               1230
Glu Ser Ile Asn Lys Ala Met Ile Asn Ile Asn Lys Phe Leu Asn Gln
 1235                               1240               1245
Cys Ser Val Ser Tyr Leu Met Asn Ser Met Ile Pro Tyr Gly Val Lys
 1250                               1255               1260
Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr
 1265                               1270               1275               1280
Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys
                1285                               1290               1295
Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser
                1300                               1305               1310
Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr
                1315                               1320

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&lt;210&gt; 105

&lt;211&gt; 1329

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

## Li et al., Degradable Clostridial Toxins

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(1329)

&lt;223&gt; BoNT/A-BD-PAR3-Thrombin

&lt;400&gt; 105

Met	Pro	Phe	Val	Asn	Lys	Gln	Phe	Asn	Tyr	Lys	Asp	Pro	Val	Asn	Gly
1				5				10						15	
Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn	Ala	Gly	Gln	Met	Gln	Pro
			20					25					30		
Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile	Trp	Val	Ile	Pro	Glu	Arg
		35					40					45			
Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro	Glu
	50					55					60				
Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
	115					120						125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130				135						140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165				170						175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
			245					250						255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
		260						265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
	275						280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
			325					330						335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
		340						345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355				360						365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
			405					410						415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg

Li *et al.*, Degradable Clostridial Toxins

			420					425				430			
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Lys	Thr	Phe	Arg	Gly
			435					440				445			
Ala	Pro	Pro	Asn	Ser	Phe	Glu	Glu	Phe	Pro	Ala	Leu	Asn	Asp	Leu	Phe
			450					455				460			
Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg
465								470				475			480
Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile
				485											495
Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile
			500												510
Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn
			515									525			
Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp
			530					535				540			
Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr
545						550						555			560
Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu
				565											575
Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys
			580												590
Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr
			595					600				605			
Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn
			610					615				620			
Ser	Lys	Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser
625						630						635			640
Asn	Leu	Gly	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp
				645											655
Gly	Cys	Arg	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu
			660												670
Phe	Asp	Lys	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn
			675					680				685			
Gln	Ser	Asn	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln
			690					695				700			
Tyr	Asp	Lys	Pro	Tyr	Tyr	Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr
705						710						715			720
Val	Asp	Val	Asn	Asn	Val	Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly
				725											735
Pro	Arg	Gly	Ser	Val	Met	Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu
			740												750
Tyr	Arg	Gly	Thr	Lys	Phe	Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys
			755												765
Asp	Asn	Ile	Val	Arg	Asn	Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val
			770												780
Lys	Asn	Lys	Glu	Tyr	Arg	Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val
785						790						795			800
Glu	Lys	Ile	Leu	Ser	Ala	Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser
				805											815
Gln	Val	Val	Val	Met	Lys	Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys
				820											830
Cys	Lys	Met	Asn	Leu	Gln	Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile
			835												845
Gly	Phe	His	Gln	Phe	Asn	Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp
			850												860
Tyr	Asn	Arg	Gln	Ile	Glu	Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp
865						870									880
Glu	Phe	Ile	Pro	Val	Asp	Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu
				885											895

## Li et al., Degradable Clostridial Toxins

Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 900 905 910  
 Ala Leu Val Leu Gln Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe  
 915 920 925  
 Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu  
 930 935 940  
 Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu  
 945 950 955 960  
 Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro  
 965 970 975  
 Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu  
 980 985 990  
 Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu  
 995 1000 1005  
 Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu  
 1010 1015 1020  
 His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu  
 1025 1030 1035 1040  
 Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys  
 1045 1050 1055  
 Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu  
 1060 1065 1070  
 Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr  
 1075 1080 1085  
 Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala  
 1090 1095 1100  
 Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu  
 1105 1110 1115 1120  
 Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala  
 1125 1130 1135  
 Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys  
 1140 1145 1150  
 Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu  
 1155 1160 1165  
 Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys  
 1170 1175 1180  
 Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu  
 1185 1190 1195 1200  
 Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn  
 1205 1210 1215  
 Gln Tyr Thr Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp  
 1220 1225 1230  
 Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile  
 1235 1240 1245  
 Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met  
 1250 1255 1260  
 Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys  
 1265 1270 1275 1280  
 Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly  
 1285 1290 1295  
 Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp  
 1300 1305 1310  
 Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser  
 1315 1320 1325  
 Thr

Li *et al.*, Degradable Clostridial Toxins

<211> 1323  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> PEPTIDE  
 <222> (1)...(1323)  
 <223> BoNT/A-BD-PAR3-Xa

<400> 106

Met	Pro	Phe	Val	Asn	Lys	Gln	Phe	Asn	Tyr	Lys	Asp	Pro	Val	Asn	Gly
1				5					10					15	
Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn	Ala	Gly	Gln	Met	Gln	Pro
			20					25					30		
Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile	Trp	Val	Ile	Pro	Glu	Arg
		35					40					45			
Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro	Glu
	50					55					60				
Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115					120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165					170					175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230				235						240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
			245						250					255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
		275					280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
				325					330					335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn

## Li et al., Degradable Clostridial Toxins

385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
				405					410					415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Thr	Phe
		435					440					445			
Arg	Gly	Ala	Pro	Ala	Leu	Asn	Asp	Leu	Phe	Thr	Glu	Tyr	Ile	Lys	Asn
	450					455					460				
Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu
465					470					475					480
Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val
				485					490					495	
Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu
			500					505					510		
Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser
		515					520					525			
Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr
	530					535					540				
Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met
545					550				555						560
Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile
				565					570					575	
Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys
			580					585					590		
Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe
		595					600					605			
Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn
	610					615					620				
Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His
625					630					635					640
Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr	His
				645					650					655	
Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu	Asn
			660					665					670		
Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly	Ile
		675					680					685			
Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr	Tyr
	690					695					700				
Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn	Val
705					710					715					720
Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val	Met
				725					730					735	
Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys	Phe
			740					745					750		
Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg	Asn
		755					760					765			
Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr	Arg
	770					775					780				
Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val	Glu	Lys	Ile	Leu	Ser	Ala
785					790					795					800
Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser	Gln	Val	Val	Val	Met	Lys
				805					810					815	
Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys	Cys	Lys	Met	Asn	Leu	Gln
			820					825					830		
Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile	Gly	Phe	His	Gln	Phe	Asn
	835					840					845				
Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp	Tyr	Asn	Arg	Gln	Ile	Glu
	850					855					860				

## Li et al., Degradable Clostridial Toxins

Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp	Glu	Phe	Ile	Pro	Val	Asp
865					870					875					880
Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu	Ala	Gly	Gly	Gly	Gly	Ser
				885					890						895
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Leu	Val	Leu	Gln	Cys
			900					905					910		
Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn
	915					920						925			
Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn
	930					935					940				
Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr
945					950					955					960
Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu
			965					970						975	
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile
			980				985						990		
Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met
	995					1000						1005			
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile
	1010					1015					1020				
Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val
1025					1030					1035					1040
Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr
			1045					1050						1055	
Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe
			1060				1065						1070		
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile
	1075					1080						1085			
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met
	1090					1095					1100				
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val
1105					1110					1115					1120
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr
			1125					1130						1135	
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr
			1140					1145					1150		
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr
	1155					1160						1165			
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp
	1170					1175					1180				
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala
1185					1190					1195					1200
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu
			1205					1210					1215		
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn
			1220					1225					1230		
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln
			1235				1240					1245			
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys
	1250					1255				1260					
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr
1265					1270					1275					1280
Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys
			1285					1290						1295	
Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser
			1300					1305					1310		
Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr					
	1315						1320								



## Li et al., Degradable Clostridial Toxins

<210> 107  
 <211> 1329  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> PEPTIDE  
 <222> (1)...(1329)  
 <223> BoNT/A-BD-PAR4-Thrombin

<400> 107  
 Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
 1 5 10 15  
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro  
 20 25 30  
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
 35 40 45  
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu  
 50 55 60  
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr  
 65 70 75 80  
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu  
 85 90 95  
 Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val  
 100 105 110  
 Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys  
 115 120 125  
 Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr  
 130 135 140  
 Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile  
 145 150 155 160  
 Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr  
 165 170 175  
 Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe  
 180 185 190  
 Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu  
 195 200 205  
 Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu  
 210 215 220  
 Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn  
 225 230 235 240  
 Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu  
 245 250 255  
 Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys  
 260 265 270  
 Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn  
 275 280 285  
 Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val  
 290 295 300  
 Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys  
 305 310 315 320  
 Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu  
 325 330 335  
 Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp  
 340 345 350  
 Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn  
 355 360 365  
 Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr

## Li et al., Degradable Clostridial Toxins

370	375	380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn		
385	390	395
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu		400
	405	410
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg		415
	420	425
Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Pro Arg Gly Tyr Pro Gly		430
	435	440
Gln Val Cys Ala Asn Asp Ser Asp Thr Leu Ala Leu Asn Asp Leu Phe		445
	450	455
Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn Leu Arg		460
465	470	475
Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser Lys Ile		480
	485	490
Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn Gln Ile		495
	500	505
Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn		510
	515	520
Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp		525
	530	535
Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn Glu Tyr		540
545	550	555
Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu		560
	565	570
Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu Ile Lys		575
	580	585
Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr		590
	595	600
Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn		605
	610	615
Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser		620
625	630	635
Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp		640
	645	650
Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu		655
	660	665
Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn		670
	675	680
Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln		685
	690	695
Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr		700
705	710	715
Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly		720
	725	730
Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu		735
	740	745
Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys		750
	755	760
Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val		765
	770	775
Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val		780
785	790	795
Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser		800
	805	810
Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys		815
	820	825
Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile		830
	835	840
		845

## Li et al., Degradable Clostridial Toxins

Gly	Phe	His	Gln	Phe	Asn	Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp	850	855	860
Tyr	Asn	Arg	Gln	Ile	Glu	Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp	865	870	875
Glu	Phe	Ile	Pro	Val	Asp	Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu	885	890	895
Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	900	905	910
Ala	Leu	Val	Leu	Gln	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	915	920	925
Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	930	935	940
Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Asn	Ile	Ser	Leu		945	950	955
Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	965	970	975
Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	980	985	990
Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	995	1000	1005
Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	1010	1015	1020
His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	1025	1030	1035
Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	1045	1050	1055
Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	1060	1065	1070
Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	1075	1080	1085
Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	1090	1095	1100
Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	1105	1110	1115
Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	1125	1130	1135
Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	1140	1145	1150
Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	1155	1160	1165
Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	1170	1175	1180
Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	1185	1190	1195
Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	1205	1210	1215
Gln	Tyr	Thr	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp		1220	1225	1230
Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	1235	1240	1245
Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	1250	1255	1260
Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	1265	1270	1275
Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	1285	1290	1295
Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	1300	1305	1310
Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser			

Li *et al.*, Degradable Clostridial Toxins

1315                      1320                      1325  
 Thr  
  
 <210> 108  
 <211> 1323  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <221> PEPTIDE  
 <222> (1)...(1323)  
 <223> BoNT/A-BD-PAR4-Xa  
  
 <400> 108  
 Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
 1                      5                      10                      15  
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro  
 20                      25                      30  
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
 35                      40                      45  
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu  
 50                      55                      60  
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr  
 65                      70                      75                      80  
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu  
 85                      90                      95  
 Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val  
 100                      105                      110  
 Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys  
 115                      120                      125  
 Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr  
 130                      135                      140  
 Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile  
 145                      150                      155                      160  
 Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr  
 165                      170                      175  
 Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe  
 180                      185                      190  
 Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu  
 195                      200                      205  
 Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu  
 210                      215                      220  
 Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn  
 225                      230                      235                      240  
 Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu  
 245                      250                      255  
 Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys  
 260                      265                      270  
 Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn  
 275                      280                      285  
 Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val  
 290                      295                      300  
 Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys  
 305                      310                      315                      320  
 Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu  
 325                      330                      335  
 Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp

## Li et al., Degradable Clostridial Toxins

			340				345				350				
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
			355				360				365				
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
			370				375				380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385				390				395				400			
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
			405				410				415				
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420				425				430				
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Gly	Tyr
			435				440				445				
Pro	Gly	Gln	Val	Ala	Leu	Asn	Asp	Leu	Phe	Thr	Glu	Tyr	Ile	Lys	Asn
			450				455				460				
Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu
465				470				475				480			
Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val
			485				490				495				
Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu
			500				505				510				
Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser
			515				520				525				
Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr
			530				535				540				
Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met
545				550				555				560			
Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile
			565				570				575				
Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys
			580				585				590				
Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe
			595				600				605				
Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn
			610				615				620				
Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His
625				630				635				640			
Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr	His
			645				650				655				
Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu	Asn
			660				665				670				
Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly	Ile
			675				680				685				
Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr	Tyr
			690				695				700				
Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn	Val
705				710				715				720			
Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val	Met
			725				730				735				
Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys	Phe
			740				745				750				
Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg	Asn
			755				760				765				
Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr	Arg
			770												

## Li et al., Degradable Clostridial Toxins

				805					810					815			
Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys	Cys	Lys	Met	Asn	Leu	Gln		
			820					825					830				
Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile	Gly	Phe	His	Gln	Phe	Asn		
		835					840					845					
Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp	Tyr	Asn	Arg	Gln	Ile	Glu		
	850					855					860						
Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp	Glu	Phe	Ile	Pro	Val	Asp		
865					870					875					880		
Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu	Ala	Gly	Gly	Gly	Gly	Ser		
			885						890						895		
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Leu	Val	Leu	Gln	Cys		
		900						905					910				
Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn		
	915					920						925					
Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn		
	930					935					940						
Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr		
945					950					955					960		
Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu		
			965					970						975			
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile		
		980					985						990				
Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met		
	995					1000						1005					
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile		
	1010					1015					1020						
Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val		
1025					1030						1035				1040		
Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr		
			1045						1050					1055			
Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe		
		1060						1065					1070				
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile		
	1075						1080					1085					
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met		
	1090					1095					1100						
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val		
1105					1110					1115					1120		
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr		
			1125						1130					1135			
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr		
		1140						1145					1150				
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr		
	1155					1160						1165					
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp		
	1170					1175					1180						
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala		
1185					1190					1195					1200		
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu		
			1205					1210					1215				
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn		
		1220						1225					1230				
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln		
	1235					1240						1245					
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys		
	1250					1255					1260						
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr		

Li *et al.*, Degradable Clostridial Toxins

1265		1270		1275		1280
Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys						
		1285		1290		1295
Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser						
		1300		1305		1310
Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr						
		1315		1320		

&lt;210&gt; 109

&lt;211&gt; 4053

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4050)

&lt;223&gt; Sequence encoding BoNT/A-ED-PAR1-Thrombin

&lt;400&gt; 109

```

atggggccgc ggccgctgct gctggtggcc gcctgcttca gtctgtgcgg cccgctggtg 60
tctgcccgc cccgggccc caggccagaa tcaaaagcaa caaatgccac cttagatccc 120
cggtcatttc ttctcaggaa cccaatgat aaatatgaac catttccatt tgtaataaaa 180
caatttaatt ataaagatcc tgtaaatggt gttgatattg cttatataaa aattccaaat 240
gcaggacaaa tgcaaccagt aaaagctttt aaaattcata ataaaatatg gggtattcca 300
gaaagagata catttacaaa tcctgaagaa ggagatttaa atccaccacc agaagcaaaa 360
caagttccag ttcatatta tgattcaaca tatttaagta cagataatga aaaagataat 420
tatttaaagg gagttacaaa attatttgag agaatttatt caactgatct tgggaagaatg 480
ttgttaacat caatagtaag gggaatacca ttttggggtg gaagtacaat agatacagaa 540
ttaaaagtta ttgatactaa ttgtattaat gtgatacaac cagatggtag ttatagatca 600
gaagaactta atctagtaat aataggaccc tcagctgata ttatacagtt tgaatgtaaa 660
agctttggac atgaagtttt gaatcttacg cgaaatggtt atggctctac tcaatacatt 720
agatttagcc cagattttac atttggtttt gaggagtcac ttgaagttga tacaatcct 780
cttttaggtg caggcaaatt tgctacagat ccagcagtaa cattagcaca tgaacttata 840
catgctggac atagattata tggaatagca attaatccaa atagggtttt taaagtaaat 900
actaatgcct attatgaaat gagtgggtta gaagtaagct ttgaggaact tagaacattt 960
gggggacatg atgcaaagtt tatagatagt ttacaggaaa acgaatttcg tctatattat 1020
tataataagt ttaaagatat agcaagtaca cttaataaag ctaaatcaat agtaggtact 1080
actgcttcat tacagtatat gaaaaatgtt ttaaagaga aatatctcct atctgaagat 1140
acatctggaa aattttcggg agataaatta aaatttgata agttatacaa aatgttaaca 1200
gagatttaca cagaggataa ttttgtttaag ttttttaaag tacttaacag aaaaacatat 1260
ttgaattttg ataaagccgt atttaagata aatatagtag ctaaggtaaa ttacacaata 1320
tatgatggat ttaatttaag aaatacaaat ttagcagcaa actttaatgg tcaaaaataca 1380
gaaattaata atatgaattt tactaaacta aaaaatttta ctggattgtt tgaattttat 1440
aagttgctat gtgtaagagg gataataact tctaaaacta aatcattaga taaaggatac 1500
aataaggcat taaatgattt atgtatcaaa gttaataatt gggacttggt ttttagtcct 1560
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caattagaac ttatgcctaa tatagaaaga tttcctaata gaaaaaagta tgagtttagat 1800
aaatatacta tgttccatta tcttcgtgct caagaatttg aacatggtaa atctaggatt 1860
gctttaacaa attctgttaa cgaagcatta ttaaataccta gtcgtgttta tacatttttt 1920
tcttcagact atgtaaagaa agttaataaaa gctacggagg cagctatggt tttaggctgg 1980
gtagaacaat tagtatatga ttttaccgat gaaactagcg aagtaagtac tacggataaa 2040
attgcggaata taactataat tattccatat ataggacctg ctttaaatat aggtaatatg 2100
ttatataaag atgattttgt aggtgcttta atattttcag gagctgttat tctgttagaa 2160
tttataccag agattgcaat acctgtatta ggtacttttg cacttgatc atatatgctg 2220
aataagggtt taaccgttca aacaatagat aatgctttta gtaaaagaaa tgaaaaatgg 2280
gatgaggtct ataaatatat agtaacaaat tgggttagcaa aggttaatac acagattgat 2340

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Li *et al.*, Degradable Clostridial Toxins

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ctaataagaa aaaaaatgaa agaagcttta gaaaatcaag cagaagcaac aaaggctata 2400
ataaactatc agtataatca atatactgag gaagagaaaa ataataattaa ttttaataatt 2460
gatgatttaa gttcgaact taatgagtct ataaataaag ctatgattaa tataaataaaa 2520
tttttgaatc aatgctctgt ttcataattta atgaattcta tgatccctta tgggtgttaa 2580
cgggttagaag attttgatgc tagtcttaaa gatgcattat taaagtatat atatgataat 2640
agaggaactt taattgggtca agtagataga ttaaaagata aagttaataa tacacttagt 2700
acagatatac cttttcagct ttccaaatac gtagataatc aaagattatt atctacattt 2760
actgaatata ttaagaatat tattaatact tctataattga atttaagata tgaaagtaat 2820
catttaatag acttatctag gtatgcatca aaaaataaata ttggtagtaa agtaaatttt 2880
gatccaatag ataaaaatca aattcaatta ttttaatttag aaagtagtaa aattgaggta 2940
attttaaaaa atgctattgt atataatagt atgtatgaaa atttttagtac tagcttttgg 3000
ataagaattc ctaagtattt taacagtata agtctaaata atgaatatac aataataaat 3060
tgtatggaaa ataattcagg atggaaaagta tcacttaatt atggtgaaat aatctggact 3120
ttcaggata ctcaggaaat aaaacaaaga gtagttttta aatacagtca aatgattaat 3180
atatcagatt atataaacag atggattttt gtaactatca ctaataatag attaaataac 3240
tctaaaattt atataaatgg aagattaata gatcaaaaac caatttcaaa tttaggtaat 3300
attcatgcta gtaataatat aatgttttaa ttagatgggt gtagagatac acatagatat 3360
atltggataa aatatlttaa tctlttttgat aaggaattaa atgaaaaaga aatcaaagat 3420
ttatatgata atcaatcaaa ttcagggtatt ttaaaagact tttgggggtga ttattttacaa 3480
tatgataaac catactatat gttaaattta tatgatccaa ataaatatgt cgatgtaaat 3540
aatgtaggta ttagagggtta tatgtatctt aaagggccta gaggttagcgt aatgactaca 3600
aacattttatt taaattcaag tttgtatagg gggacaaaat ttattataaa aaaatatgct 3660
tctggaaata aagataatat tgtagaaat aatgatcgtg tatataattaa tgtagtagtt 3720
aaaaataaag aatatagggt agctactaat gcgtcacagg caggcgtaga aaaaatacta 3780
agtgcattag aaataacctga tgtaggaaat ctaagtcaag tagtagtaat gaagtcaaaa 3840
aatgatcaag gaataacaaa taaatgcaaa atgaatttac aagataataa tgggaatgat 3900
ataggcttta taggatttca tcagtttaat aatatagcta aactagtagc aagtaattgg 3960
tataatagac aaatagaaag atctagtagg actttgggtt gctcatggga atttattcct 4020
gtagatgatg gatggggaga aaggccactg taa 4053

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&lt;210&gt; 110

&lt;211&gt; 4029

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4026)

&lt;223&gt; Sequence encoding BoNT/A-ED-PAR1-Xa

&lt;400&gt; 110

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atggggccgc ggcggctgct gctgggtggcc gcctgcttca gtctgtgcgg cccgctgttg 60
tctgcccgca cccgggcccgc caggccagaa tcaaaagcaa caaatgccac catagaaggc 120
cggtcatttc ttctcaggaa cccatttgtt aataaacaat ttaattataa agatcctgta 180
aatgggtgttg atattgctta tataaaaaatt ccaaatgcag gacaaatgca accagtaaaa 240
gcttttaaaa ttcataataa aatatgggtt attccagaaa gagatacatt tacaaatcct 300
gaagaaggag atttaaatcc accaccagaa gcaaaacaag ttccagtttc atattatgat 360
tcaacatatt taagtacaga taatgaaaaa gataattatt taaagggagt tacaaaaatta 420
tttgagagaa ttatttcaac tgatcttgga agaattgtgt taacatcaat agtaagggga 480
ataccatttt ggggtggaag tacaatagat acagaattaa aagttattga tactaattgt 540
attaatgtga tacaaccaga tggtagttat agatcagaag aacttaatct agtaataata 600
ggaccctcag ctgatattat acagtttgaa tgtaaaagct ttggacatga agttttgaat 660
cttacgcgaa atgggttatgg ctctactcaa tacattagat ttagcccaga ttttacattt 720
ggttttgagg agtcacttga agttgataca aatcctcttt taggtgcagg caaatttgct 780
acagatccag cagtaacatt agcacatgaa cttatacatg ctggacatag atttatatgga 840
atagcaatta atccaaatag ggttttttaa gtaaatacta atgcctatta tgaaatgagt 900
gggttagaag taagctttga ggaacttaga acatttgggg gacatgatgc aaagtttata 960
gatagtttac aggaaaacga atttcgtcta tattattata ataagtttaa agatatagca 1020
agtacactta ataaagctaa atcaatagta ggtactactg cttcattaca gtatatgaaa 1080

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Li *et al.*, Degradable Clostridial Toxins

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aatgtttttta aagagaaata tctcctatct gaagatacat ctggaaaatt ttcggtagat 1140
aaattaaaaat ttgataagtt atacaaaatg ttaacagaga tttacacaga ggataatttt 1200
gttaagttttt ttaaagtact taacagaaaa acatatttga attttgataa agccgtattt 1260
aagataaata tagtacctaa ggtaaattac acaatatatg atggatttaa ttaagaaat 1320
acaaatttag cagcaaactt taatggtcaa aatacagaaa ttaataatat gaattttact 1380
aaactaaaaa attttactgg attgtttgaa ttttataagt tgctatgtgt aagagggata 1440
ataacttcta aaactaaatc attagataaa ggatacaata aggcatataa tgatttatgt 1500
atcaaagtta ataattggga cttgtttttt agtccttcag aagataattt tactaatgat 1560
ctaaataaag gagaagaaat tacatctgat actaatatag aagcagcaga agaaaaatatt 1620
agtttagatt taatacaaca atattattta acctttaatt ttgataatga acctgaaaaat 1680
atttcaatag aaaatctttc aagtgcacat ataggccaat tagaacttat gcctaataata 1740
gaaagatttc ctaattggaaa aaagtatgag ttagataaat atactatgtt ccattatctt 1800
cgtgctcaag aatttgaaca tggtaaatct aggattgctt taacaaattc tgtaacgaa 1860
gcattattaa atcctagtctg tgtttataca tttttttctt cagactatgt aaagaaagtt 1920
aataaagcta cggaggcagc tatgttttta ggctgggtag aacaattagt atatgatttt 1980
accgatgaaa ctagcgaagt aagtactacg gataaaattg cggatataac tataattatt 2040
ccatatatag gacctgcttt aaatataggt aatatgttat ataaagatga tttttagagg 2100
gctttaatat tttcaggagc tgttattctg ttagaattta taccagagat tgcaatacct 2160
gtattaggta cttttgcact tgtatcatat attgcgaata aggttctaac cgttcaaaca 2220
atagataatg ctttaagtaa aagaaatgaa aaatgggatg aggtctataa atatatagta 2280
acaaattggt tagcaaaggt taatacacag attgatctaa taagaaaaaa aatgaaagaa 2340
gcttttagaaa atcaagcaga agcaacaaag gctataataa actatcagta taatcaatat 2400

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actgaggaag agaaaaataa tattaatttt aatattgatg atttaagttc gaaacttaat 2460
gagtctataa ataaagctat gattaatata aataaatttt tgaatcaatg ctctgtttca 2520
tatttaatat attctatgat cccttatggg gttaaaccgg tagaagattt tgatgctagt 2580
cttaaagatg cagtattaaa gtatatatat gataatagag gaactttaat tgggtcaagta 2640
gatagattaa aagataaagt taataatata cttagtagag atataccttt tcagctttcc 2700
aaatacgtag ataatacaag attattatct acatttactg aatatattaa gaatattatt 2760
aatacttcta tattgaattt aagatatgaa agtaatcatt taatagactt atctagggtat 2820
gcatcaaaaa taaatatttg tagtaaaagt aattttgatc caatagataa aaatcaaatt 2880
caattatttt atttagaag tagtaaaatt gaggtaatth taaaaaatgc tattgtatat 2940
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caaagagtag tttttaaata cagtcaaag attaatatat cagattatat aaacagatgg 3180
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tttgataagg aattaaatga aaaagaaatc aaagatttat atgataatca atcaaattca 3420
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aattttatag atccaaata ataggtcgat gtaataaatg taggtattag aggttatatg 3540
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tgcaaaatga atttacaaga taataatggg aatgatatag gctttatagg atttcatcag 3900
tttaataata tagctaaact agtagcaagt aattggtata atagacaaat agaaagatct 3960
agtaggactt tgggttgctc atgggaattt attcctgtag atgatggatg gggagaaagg 4020
ccactgtaa 4029

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&lt;210&gt; 111

&lt;211&gt; 4038

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

Li *et al.*, Degradable Clostridial Toxins

&lt;222&gt; (1)...(4035)

&lt;223&gt; Sequence encoding BoNT/A-ED-PAR2-Trypsin

&lt;400&gt; 111

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atgcggagcc ccagcgcggc gtggctgctg ggggccgcc tctgctagc agcctctctc 60
tcctgcagtg gcaccatcca aggaaccaat agatcctcta aaggaagaag cttatttggg 120
aagggttgatg gcacatccca cgtcactgga ccatttggtta ataaacaatt taattataaa 180
gatcctgtaa atgggtgttg tattgcttat ataaaaattc caaatgcagg acaaatgcaa 240
ccagtaaaag cttttaaaat tcataataaa atatgggtta ttccagaaag agatacattt 300
acaaatcctg aagaaggaga tttaaattcca ccaccagaag caaaacaagt tccagtttca 360
tattatgatt caacatattt aagtacagat aatgaaaaag ataattattt aaaggagggt 420
acaaaattat ttgagagaat ttattcaact gatcttgga gaattgtgtt aacatcaata 480
gtaaggggaa taccattttg ggggtggaagt acaatagata cagaattaaa agttattgat 540
actaattgta ttaatgtgat acaaccagat ggtagttata gatcagaaga acttaattcta 600
gtaataatag gaccctcagc tgatattata cagtttgaat gtaaaagctt tggacatgaa 660
gttttgaatc ttacgcgaaa tggttatggc tctactcaat acattagatt tagcccatg 720
tttacatttg gttttgagga gtcacttgaa gttgatatac atcctctttt aggtgcaggc 780
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ttatatggaa tagcaattaa tccaaatagg gtttttaaag taaatactaa tgcctattat 900
gaaatgagtg ggtagaagt aagctttgag gaacttagaa catttggggg acatgatgca 960

aagtttatag atagtttaca ggaaaacgaa tttcgtctat attattataa taagttttaa 1020
gatatagcaa gtacacttaa taaagctaaa tcaatagtag gtactactgc ttcattacag 1080
tatatgaaaa atgtttttta agagaaatat ctctatctg aagatacatc tggaaaattt 1140
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gataattttg ttaagttttt taaagtactt aacagaaaaa catatttgaa ttttgataaa 1260
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aatcaatata ctgaggaaga gaaaaataat attaatttta atattgatga ttttaagttcg 2460
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tctgtttcat atttaatgaa ttctatgatc ctttatgggt ttaaaccggt agaagatttt 2580
gatgctagtc ttaaagatgc attattaaag tatatatatg ataataagag aactttaatt 2640
ggatcaagtag atagattaaa agataaagtt aataatacac ttagtacaga tatacctttt 2700
cagctttcca aatacgtaga taatcaaaga ttattatcta catttactga atatattaag 2760
aatattatta atacttctat attgaattta agatatgaaa gtaatcattt aatagactta 2820
tctaggtatg catcaaaaat aaatattgggt agtaaagtaa attttgatcc aatagataaa 2880

aatcaaatc aattatttaa tttagaaagt agtaaaattg aggtaatttt aaaaaatgct 2940
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tattttaaca gtataagtct aaataatgaa tatacaataa taaattgtat ggaaaaataa 3060
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Li *et al.*, Degradable Clostridial Toxins

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aacagatgga tttttgtaac tatcactaat aatagattaa ataactctaa aatttatata 3240
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aatataatgt ttaaattaga tgggtgtaga gatacacata gatataattg gataaaatat 3360
tttaatcttt ttgataagga attaaatgaa aaagaaatca aagatttata tgataatcaa 3420
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tatatgttaa atttatatga tccaaataaa tatgtcgtatg taaataatgt aggtattaga 3540
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aatattgtta gaaataatga tcgtgtatat attaatgtag tagttaaaaa taaagaatat 3720
aggtttagcta ctaatgcgtc acaggcaggc gtagaaaaaa tactaagtgc attagaaata 3780
cctgatgtag gaaatctaag tcaagtagta gtaatgaagt caaaaaatga tcaaggaata 3840
acaaataaat gcaaaatgaa tttacaagat aataatggga atgatatagg ctttatagga 3900
tttcatcagt ttaataatat agctaaacta gtagcaagta attggtataa tagacaaata 3960
gaaagatcta gtaggacttt ggggtgctca tgggaattta ttcctgtaga tgatggatgg 4020
ggagaaaggc cactgtaa                                     4038

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&lt;210&gt; 112

&lt;211&gt; 4014

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4011)

&lt;223&gt; Sequence encoding BoNT/A-ED-PAR2-Xa

&lt;400&gt; 112

```

atgcgagagc ccagcgcggc gtggctgctg ggggcgcgcca tcctgctagc agcctctctc 60
tcctgcagtg gcaccatcca aggaaccaat agatccatag aaggaagaag ctttattggg 120
aaggttccat ttgttaataa acaattttaat tataaagatc ctgtaaatgg tgttgatatt 180
gcttatataa aaattccaaa tgcaggacaa atgcaaccag taaaagcttt taaaattcat 240
aataaaatat gggttattcc agaaagagat acatttacia atcctgaaga aggagattta 300
aatccaccac cagaagcaaa acaagttcca gtttcatatt atgattcaac atattttaagt 360
acagataatg aaaaagataa ttattttaaag ggagttacaa aattatttga gagaatttat 420
tcaactgata ttggaagaat gttgttaaca tcaatagtaa ggggaatacc attttgggg 480
ggaagtacaa tagatacaga attaaaagtt attgatacta attgtattaa tgtgatacaa 540
ccagatggta gttatagatc agaagaactt aatctagtaa taataggacc ctcagctgat 600
attatacagt ttgaatgtaa aagcttttga catgaagttt tgaatcttac gcgaaatgg 660
tatggctcta ctcaatacat tagattttagc ccagatttta catttggttt tgaggagtca 720
cttgaagttg atacaaatcc tcttttaggt gcaggcaaat ttgctacaga tccagcagta 780
acattagcac atgaacttat acatgcttga catagattat atggaatagc aattaatcca 840
aatagggttt ttaaagtaaa tactaatgcc tattatgaaa tgagtgggtt agaagtaagc 900
tttgagggaac ttagaacatt tgggggacat gatgcaaagt ttatagatag tttacaggaa 960
aacgaatttc gtctatatta ttataataag tttaaagata tagcaagtac acttaataaa 1020
gctaaatcaa tagtaggtac tactgcttca ttacagtata tgaaaaatgt ttttaaagag 1080
aaatatctcc tatctgaaga tacatctgga aaattttcgg tagataaatt aaaatttgat 1140
aagttataca aaatgttaac agagattttac acagaggata attttgttaa gttttttaaa 1200
gtacttaaca gaaaaacata tttgaatttt gataaagccg tatttaagat aaatatagta 1260
cctaaggtaa attacacaat atatgatgga ttttaattta gaaatacaaa tttagcagca 1320
aactttaatg gtcaaaatac agaaattaat aatatgaatt ttactaaact aaaaaatttt 1380
actggattgt ttgaatttta taagttgcta tgtgtaagag ggataataac ttctaaaact 1440
aatcatttag ataaaggata caataaggca ttaaattgatt tatgtatcaa agttaataat 1500
tgggacttgt ttttttagtcc ttcagaagat aattttacta atgatctaaa taaaggagaa 1560
gaaattacat ctgatactaa tatagaagca gcagaagaaa atattagttt agattttaata 1620
caacaatatt atttaacctt taattttgat aatgaacctg aaaatatttc aatagaaaat 1680
ctttcaagtg acattatagg ccaattagaa cttatgccta atatagaaaag atttccta 1740
ggaaaaaagt atgagttaga taaatatact atgttccatt atcttcgtgc tcaagaattt 1800
gaacatggta aatctaggat tgctttaaca aattctgtta acgaagcatt attaaatcct 1860

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## Li et al., Degradable Clostridial Toxins

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agtcgtgttt  atacattttt  ttcttcagac  tatgtaaaga  aagttaataa  agctacggag  1920
gcagctatgt  ttttaggctg  ggtagaacia  ttagtatatg  attttaccga  tgaaactagc  1980
gaagtaagta  ctacggataa  aattgcggat  ataactataa  ttattccata  tataggacct  2040
gctttaaata  taggtaatat  gttatataaa  gatgattttg  taggtgcttt  aatattttca  2100
ggagctgtta  ttctgttaga  atttatacca  gagattgcaa  tacctgtatt  aggtactttt  2160
gcacttgat  catatattgc  gaataagggt  ctaaccgttc  aaacaataga  taatgcttta  2220
agtaaaagaa  atgaaaaatg  ggatgagggt  tataaatata  tagtaacaaa  ttgggttagca  2280
aaggtttaata  cacagattga  tctaataaga  aaaaaaatga  aagaagcttt  agaaaatcaa  2340
gcagaagcaa  caaaggctat  aataaactat  cagtataatc  aatatactga  ggaagagaaa  2400
aataatatta  attttaatat  tgatgattta  agttcgaaac  ttaatgagtc  tataaataaa  2460
gctatgatta  atataaataa  atttttgaat  caatgctctg  ttcatatttt  aatgaattct  2520
atgatccctt  atgggtgtta  acgggttagaa  gattttgatg  ctagtcttaa  agatgcatta  2580
ttaaagtata  tatatgataa  tagaggaact  ttaattgggt  aagtagatag  attaaaagat  2640
aaagtttaata  atacacttag  tacagatata  ctttttcagc  tttccaaata  cgtagataat  2700
caaagattat  tatctacatt  tactgaatat  attaagaata  ttattaatac  ttctatattg  2760
aatttaagat  atgaaagtaa  tcatttaata  gacttatcta  ggtatgcac  aaaaaataat  2820
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gaaagtagta  aaattgaggt  aattttaaaa  aatgctattg  tatataatag  tatgtatgaa  2940
aatttttagta  ctagcttttg  gataagaatt  cctaagtatt  ttaacagtat  aagtctaaat  3000
aatgaatata  caataataaa  ttgtatggaa  aataattcag  gatggaaagt  atcacttaat  3060
tatgggtgaa  taatctggac  tttacaggat  actcaggaaa  taaaacaaag  agtagttttt  3120
aaatacagtc  aaatgattaa  tatatcagat  tatataaaca  gatggatttt  tgtaactatc  3180
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tgtagagata  cacatagata  tatttgggata  aaatatttta  atctttttga  taaggaatta  3360
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caagataata  atgggaatga  tataggcttt  ataggatttc  atcagtttaa  taatatagct  3900
aaactagtag  caagtaattg  gtataataga  caaatagaaa  gatctagtag  gactttgggt  3960
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&lt;210&gt; 113

&lt;211&gt; 4044

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4041)

&lt;223&gt; Sequence encoding BoNT/A-ED-PAR3-Thrombin

&lt;400&gt; 113

```

atgaaagccc  tcatctttgc  agctgctggc  ctctgcttc  tgttgccac  tttttgtcag  60
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cgtggagctc  ccccaaattc  ttttgaagag  ttcccccat  ttgttaataa  acaatttaat  180
tataaagatc  ctgtaaatgg  tgttgatatt  gcttatataa  aaattccaaa  tgcaggacaa  240
atgcaaccag  taaaagcttt  taaaattcat  aataaaatat  gggttattcc  agaaagagat  300
acatttacaa  atcctgaaga  aggagattta  aatccaccac  cagaagcaaa  acaagttcca  360
gtttcatatt  atgattcaac  atatttaagt  acagataatg  aaaaagataa  ttattttaag  420
ggagttacaa  aattatttga  gagaatttat  tcaactgatc  ttggaagaat  gttgttaaca  480
tcaatagtaa  ggggaatacc  attttggggg  ggaagtacaa  tagatacaga  attaaaagtt  540
attgatacta  attgtattaa  tgtgatacaa  ccagatggta  gttatagatc  agaagaactt  600
aatctagtaa  taataggacc  ctgagctgat  attatacagt  ttgaatgtaa  aagctttgga  660

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Li *et al.*, Degradable Clostridial Toxins

catgaagttt	tgaatcttac	gcgaaatggt	tatggctcta	ctcaatacat	tagatttagc	720
ccagatttta	catttggttt	tgaggagtca	cttgaagttg	atacaaatcc	tcttttaggt	780
gcaggcaa	ttgctacaga	tccagcagta	acattagcac	atgaacttat	acatgctgga	840
catagattat	atggaatagc	aattaatcca	aatagggttt	ttaaagtaaa	tactaatgcc	900
tattatgaaa	tgagtgggtt	agaagtaagc	tttgagggaac	ttagaacatt	tggggggacat	960
gatgcaaagt	ttatagatag	tttacaggaa	aacgaatttc	gtctatatta	ttataataag	1020
tttaaagata	tagcaagtac	acttaataaa	gctaaatcaa	tagtaggtac	tactgcttca	1080
ttacagtata	tgaaaaatgt	ttttaaagag	aaatatctcc	tatctgaaga	tacatctgga	1140
aaattttcgg	tagataaatt	aaaatttgat	aagttataca	aaatgttaac	agagatttca	1200
acagaggata	attttggttaa	gtttttttaa	gtacttaaca	gaaaaacata	tttgaatttt	1260
gataaagccg	tattttaagat	aaatatagta	cctaaggtaa	attacacaat	atatgatgga	1320
tttaatttaa	gaaatacaaa	tttagcagca	aactttaatg	gtcaaaatac	agaaattaat	1380
aatatgaatt	ttactaaact	aaaaaatttt	actggattgt	ttgaatttta	taagttgcta	1440
tgtgtaagag	ggataataac	ttctaaaact	aaatcattag	ataaaggata	caataaggca	1500
ttaaatgatt	tatgtatcaa	agttaataat	tgggacttgt	tttttagtcc	ttcagaagat	1560
aattttacta	atgatctaaa	taaaggagaa	gaaattacat	ctgatactaa	tatagaagca	1620
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tataaatata	tagtaacaaa	ttggtttagc	aaggtttaata	cacagattga	tctaataaga	2340
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ccttttcagc	tttccaaata	cgtagataat	caaagattat	tatctacatt	tactgaatat	2760
attaagaata	ttattaatac	ttctatattg	aattttaagat	atgaaagtaa	tcattttaata	2820
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gataaaaaatc	aaattcaatt	atttaatttt	gaaagtagta	aaattgaggt	aatttttaaaa	2940
aatgctattg	tatataatag	tatgtatgaa	aatttttagta	ctagcttttg	gataagaatt	3000
cctaagtatt	ttaacagtat	aagtctaaat	aatgaatata	caataataaa	ttgtatggaa	3060
aataattcag	gatggaaagt	atcacttaat	tatggtgaaa	taatctggac	tttacaggat	3120
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aaatattttta	atctttttga	taaggaatta	aatgaaaaag	aaatcaaaga	tttatatgat	3420
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gaatataggt	tagctactaa	tgcgtcacag	gcaggcgtag	aaaaaatact	aagtgcatta	3780
gaaataacctg	atgtaggaaa	tctaagtcaa	gtagtagtaa	tgaagtcaaa	aatgatcaa	3840
ggaataacaa	ataaatgcaa	aatgaattta	caagataata	atgggaatga	tataggcttt	3900
ataggatttc	atcagtttaa	taatatagct	aaactagtag	caagtaattg	gtataataga	3960
caaatagaaa	gatctagtag	gactttgggt	tgctcatggg	aattttattcc	tgtagatgat	4020
ggatggggag	aaaggccact	gtaa				4044

Li *et al.*, Degradable Clostridial Toxins

&lt;211&gt; 4020

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4017)

&lt;223&gt; Sequence encoding BoNT/A-ED-PAR3-Xa

&lt;400&gt; 114

```

atgaaagccc tcactcttgc agctgctggc ctccctgcttc tgttgccac tttttgtcag 60
agtggcatgg aaaatgatac aaacaacttg gcaaagccaa ccatagaagg tagaaccttt 120
cgtggagctc ccccatcttg taataaacia tttaattata aagatcctgt aaatgggtgt 180
gatattgctt atataaaaaat tccaaatgca ggacaaatgc aaccagtaaa agctttttaa 240
attcataata aaatatgggt tattccagaa agagatacat ttacaaatcc tgaagaagga 300
gatttaaatc caccaccaga agcaaaaaca gttccagttt catattatga ttcaacatat 360
ttaagtacag ataatgaaaa agataattat ttaaggaggag ttacaaaatt atttgagaga 420
atattattcaa ctgatcttgg aagaatgttg ttaacatcaa tagtaagggg aataccattt 480
tgggggtggaa gtacaataga tacagaatta aaagttattg atactaattg tattaatgtg 540
atacaaccag atggtagtta tagatcagaa gaacttaatc tagtaataat aggacctca 600
gctgatatta tacagtttga atgtaaaagc tttggacatg aagttttgaa tcttacgcga 660
aatggttatg gctctactca atacattaga tttagcccag attttacatt tgggtttgag 720
gagtcacttg aagttgatac aaatcctctt ttaggtgcag gcaaatttgc tacagatcca 780
gcagtaacat tagcacatga acttatacat gctggacata gattatatgg aatagcaatt 840
aatccaaata ggggtttttaa agtaaaatact aatgcctatt atgaaatgag tgggttagaa 900
gtaagctttg aggaacttag aacatttggg ggacatgatg caaagtttat agatagttta 960
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gctttaagta aaagaaatga aaaatgggat gaggtctata aatatatagt aacaaattgg 2280
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aatcaagcag aagcaacaaa ggctataata aactatcagt ataataata tactgaggaa 2400
gagaaaaata atattaattt taatattgat gatttaagtt cgaaacttaa tgagtctata 2460
aataaagcta tgattaatat aaataaattt ttgaatcaat gctctgtttc atatttaatt 2520
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gcattattaa agtatatata tgataataga ggaactttta ttggtcaagt agatagatta 2640
aaagataaag ttaataatac acttagtaca gatatacctt ttcagctttc caaatacgta 2700
gataatcaaa gattattatc tacatttact gaatatatta agaataattat taatacttct 2760
atattgaatt taagatatga aagtaatcat ttaatagact tatctaggta tgcataaaaa 2820
ataaatattg gtagtaaagt aaattttgat ccaatagata aaaatcaaat tcaattattt 2880
aatttagaaa gtagtaaaat tgaggtaatt ttaaaaaatg ctattgtata taatagtatg 2940

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Li *et al.*, Degradable Clostridial Toxins

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tatgaaaatt ttagtactag cttttggata agaattccta agtatttttaa cagtataagt 3000
ctaaataatg aatatacaat aataaattgt atggaaaata attcaggatg gaaagtatca 3060
cttaattatg gtgaaataat ctggacttta caggatactc aggaaataaa acaaagagta 3120
gttttttaaat acagtcaaat gattaatata tcagattata taaacagatg gattttttgta 3180
actatcacta ataatagatt aaataactct aaaatttata taaatggaag attaatagat 3240
caaaaaccaa tttcaaattt aggtaatat catgctagta ataataataat gtttaaatta 3300
gatgggttgta gagatacaca tagatatatt tggataaaat attttaatct ttttgataag 3360
gaattaaatg aaaaagaaat caaagattta tatgataatc aatcaaattc aggtatttta 3420
aaagactttt ggggtgatta tttacaatat gataaaccat actatatgtt aaatttata 3480
gatccaaata aatatgtcga tgtaataat gtaggtatta gaggttatat gtatcttaaa 3540
gggcctagag gtagcgtaat gactacaaac atttatttaa attcaagttt gtataggggg 3600
acaaaattta ttataaaaaa atagcttctt ggaaataaag ataataattgt tagaaataat 3660
gatcgtgtat atattaatgt agtagttaaa aataaagaat atagggttagc tactaatgcy 3720
tcacaggcag gcgtagaaaa aatactaagt gcattagaaa tacctgatgt aggaaatcta 3780
agtcaagtag tagtaatgaa gtcaaaaaat gatcaaggaa taacaaataa atgcaaaatg 3840
aatttacaag ataataatgg gaatgatata ggctttatag gatttcatca gtttaataat 3900
atagctaaac tagtagcaag taattggtat aatagacaaa tagaaagatc tagtaggact 3960
ttgggttgct catgggaatt tttcctgta gatgatggat ggggagaaa ggcactgtaa 4020

```

&lt;210&gt; 115

&lt;211&gt; 4071

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4068)

&lt;223&gt; Sequence encoding BoNT/A-ED-PAR4-Thrombin

&lt;400&gt; 115

```

atgtgggggc gactgtctct gtggcccctg gtgctggggg tcagcctgtc tggcggcacc 60
cagaccccca gcgtctacga cgagagcggg agcaccggag gtggtgatga cagcacgccc 120
tcaatcctgc ctgcccccg cggctaccca ggccaagtct gtgccaatga cagtgcaccc 180
ctgccatttg ttaataaaca atttaattat aaagatcctg taaatgggtg tgatattgct 240
tatataaaaa ttccaaatgc aggacaaatg caaccagtaa aagcttttaa aattcataat 300
aaaatatggg ttattccaga aagagatata tttacaaatc ctgaagaagg agattttaat 360
ccaccaccag aagcaaaaca agttccagtt tcatattatg attcaacata tttaagtaca 420
gataatgaaa aagataatta tttaaaggga gttacaaaat tatttgagag aattttattca 480
actgatcttg gaagaatggt gttaacatca atagtaaggg gaataccatt ttgggggtgga 540
agtacaatag atacagaatt aaaagttatt gatactaatt gtattaatgt gatacaacca 600
gatggtagtt atagatcaga agaacttaat ctagtaataa taggaccctc agctgatatt 660
atacagtttg aatgtaaaag ctttggacat gaagttttga atcttacgcy aaatggttat 720
ggctctactc aatacattag atttagccca gattttacat ttgggttttg ggagtcactt 780
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ttagcacatg aacttatata tgctggacat agatttatat gaatagcaat taatccaaat 900
agggttttta aagtaaatac taatgcctat tatgaaatga gtgggttaga agtaagcttt 960
gaggaactta gaacatttgg gggacatgat gcaaagttaa tagatagttt acaggaaaac 1020
gaatttcgtc tatattatta taataagttt aaagatatag caagtacact taataaagct 1080
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Li *et al.*, Degradable Clostridial Toxins

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&lt;210&gt; 116

&lt;211&gt; 4047

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4044)

&lt;223&gt; Sequence encoding BoNT/A-ED-PAR4-Xa

&lt;400&gt; 116

```

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gatacattta caaatcctga agaaggagat ttaaattccac caccagaagc aaaacaagtt 360
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## Li et al., Degradable Clostridial Toxins

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Li *et al.*, Degradable Clostridial Toxins

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 gatggatggg gagaaaggcc actgtaa 4047

<210> 117

<211> 3921

<212> DNA

<213> Artificial Sequence

<220>

<221> mat\_peptide

<222> (1)...(3918)

<223> Sequence encoding BoNT/A-TD-PAR1-Thrombin

<400> 117

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 aaaatatggg ttattccaga aagagatata ttacaaatc ctgaagaagg agatttaaat 180  
 ccaccaccag aagcaaaaca agttccagtt tcatattatg attcaacata tttaagtaca 240  
 gataatgaaa aagataatta tttaaaggga gttacaaaat tatttgagag aattttattca 300  
 actgatcttg gaagaatggt gttaacatca atagtaaggg gaataccatt ttgggggtgga 360  
 agtacaaatg atacagaatt aaaagttatt gatactaatt gtattaatgt gatacaacca 420  
 gatggtagtt atagatcaga agaacttaat ctagtaataa taggaccctc agctgatatt 480  
 atacagtttg aatgtaaaag ctttggacat gaagttttga atcttacgag aaatgggtat 540  
 ggctctactc aatacattag atttagccca gattttacat ttggttttga ggagtcactt 600  
 gaagttgata caaatcctct tttagggtgca ggcaaatttg ctacagatcc agcagtaaca 660  
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 aggtttttta aagtaaatat taatgcctat tatgaaatga gtgggttaga agtaagcttt 780  
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 aagaatatta ttaatacttc tatattgaat ttaagatatg aaagtaatca tttaatagac 2700

## Li et al., Degradable Clostridial Toxins

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&lt;400&gt; 118

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Li *et al.*, Degradable Clostridial Toxins

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```

&lt;210&gt; 119

&lt;211&gt; 3921

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3918)

&lt;223&gt; Sequence encoding BoNT/A-TD-PAR2-Trypsin

&lt;400&gt; 119

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Li *et al.*, Degradable Clostridial Toxins

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- *Li et al., Degradable Clostridial Toxins*

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 <211> 3903  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <221> mat\_peptide  
 <222> (1)...(3900)  
 <223> Sequence encoding BoNT/A-TD-PAR2-Xa

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aaaatatggg ttattccaga aagagataca ttacaaaatc ctgaagaagg agattttaat 180
ccaccaccag aagcaaaaca agttccagtt tcatattatg attcaacata tttaagtaca 240
gataatgaaa aagataatta tttaaaggga gttacaaaat tatttgagag aatttattca 300
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## Li et al., Degradable Clostridial Toxins

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&lt;210&gt; 121

&lt;211&gt; 3921

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3918)

&lt;223&gt; Sequence encoding BoNT/A-TD-PAR3-Thrombin

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Li *et al.*, Degradable Clostridial Toxins

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&lt;211&gt; 3903

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

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&lt;222&gt; (1)...(3900)

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&lt;400&gt; 122

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## Li et al., Degradable Clostridial Toxins

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Li *et al.*, Degradable Clostridial Toxins

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 <213> Artificial Sequence

<220>  
 <221> mat\_peptide  
 <222> (1)...(3918)  
 <223> Sequence encoding BoNT/A-TD-PAR4-Thrombin

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## Li et al., Degradable Clostridial Toxins

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

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&lt;223&gt; Sequence encoding BoNT/A-TD-PAR4-Xa

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Li *et al.*, Degradable Clostridial Toxins

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taa 3903

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&lt;210&gt; 125

&lt;211&gt; 3987

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3984)

&lt;223&gt; Sequence encoding BoNT/A-BD-PAR1-Thrombin

&lt;400&gt; 125

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```

## Li et al., Degradable Clostridial Toxins

```

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gtagataatc aaagattatt atctaca 3987

```

Li *et al.*, Degradable Clostridial Toxins

<210> 126  
 <211> 3969  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <221> mat\_peptide  
 <222> (1)...(3966)  
 <223> Sequence encoding BoNT/A-BD-PAR1-Xa

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 aaaatatggg ttattccaga aagagatata ttacaaaatc ctgaagaagg agattttaat 180  
 ccaccaccag aagcaaaaca agttccagtt tcatattatg attcaacata tttaagtaca 240  
 gataatgaaa aagataatta tttaaagggg gttacaaaat tatttgagag aatttattca 300  
 actgatcttg gaagaatggt gttaacatca atagtaaggg gaataccatt ttgggggtgga 360  
 agtacaatag atacagaatt aaaagttatt gatactaatt gtattaatgt gatacaacca 420  
 gatggtagtt atagatcaga agaacttaat ctagtaataa taggaccctc agctgatatt 480  
 atacagtttg aatgtaaaag ctttggacat gaagttttga atcttacgag aaatgggtat 540  
 ggctctactc aatacattag atttagccca gattttacat ttggttttga ggagtcactt 600  
 gaagttgata caaatcctct tttagggtgca ggcaaatttg ctacagatcc agcagtaaca 660  
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 aggggttttta aagtaaatac taatgcctat tatgaaatga gtgggttaga agtaagcttt 780  
 gaggaactta gaacattttg gggacatgat gcaaagttta tagatagttt acaggaaaac 840  
 gaatttcgtc tatattatta taataagttt aaagatatag caagtacact taataaagct 900  
 aaatcaatag taggtactac tgcttcatta cagtatatga aaaatgtttt taaagagaaa 960  
 tatctcctat ctgaagatac atctggaaaa ttttcggtag ataaattaaa atttgataag 1020  
 ttatacaaaa tgttaacaga gatttacaca gaggataatt ttgttaagtt ttttaaagta 1080  
 cttaacagaa aaacatatatt gaattttgat aaagccgtat ttaagataaa tatagtacct 1140  
 aaggtaaat acacaatata tgatggattt aatttaagaa atacaaattt agcagcaaac 1200  
 tttaatggtc aaaatacaga aattaataat atgaatttta ctaaactaaa aaattttact 1260  
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 gataatcaat caaattcagg tattttaaaa gacttttggg gtgattattt acaatatgat 2100  
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Li *et al.*, Degradable Clostridial Toxins

```

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tatgggtgta aacgggttaga agattttgat gctagtctta aagatgcatt attaaagtat 3840
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aatacactta gtacagatat accttttcag ctttccaaat acgtagataa tcaaagatta 3960
ttatctaca                                     3969

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&lt;210&gt; 127

&lt;211&gt; 3987

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3984)

&lt;223&gt; Sequence encoding BoNT/A-BD-PAR2-Trypsin

&lt;400&gt; 127

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Li *et al.*, Degradable Clostridial Toxins

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&lt;210&gt; 128

&lt;211&gt; 3969

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3966)

&lt;223&gt; Sequence encoding BoNT/A-BD-PAR2-Xa

&lt;400&gt; 128

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ccaccaccag aagcaaaaac agttccagtt tcatattatg attcaacata ttaagtaca 240
gataatgaaa aagataatta tttaaaggga gttacaaaat tatttgagag aatttattca 300

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Li *et al.*, Degradable Clostridial Toxins

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cttaacagaa	aaacatatatt	gaattttgat	aaagccgtat	ttaagataaa	tatagtacct	1140
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Li *et al.*, Degradable Clostridial Toxins

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<210> 129

<211> 3987

<212> DNA

<213> Artificial Sequence

<220>

<221> mat\_peptide

<222> (1)...(3984)

<223> Sequence encoding BoNT/A-BD-PAR3-Thrombin

<400> 129

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 aaaatatggg ttattccaga aagagatata ttacaaatc ctgaagaagg agatttaaat 180  
 ccaccaccag aagcaaaaca agttccagtt tcatattatg attcaacata tttaagtaca 240  
 gataatgaaa aagataatta tttaaaggga gttacaaaat tatttgagag aatttattca 300  
 actgatcttg gaagaatggt gttaacatca atagtaaggg gaataccatt ttgggggtgga 360  
 agtacaatag atacagaatt aaaagttatt gatactaatt gtattaatgt gatacaacca 420  
 gatggtagtt atagatcaga agaacttaat ctagtaataa taggaccctc agctgatatt 480  
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Li *et al.*, Degradable Clostridial Toxins

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gatttaatac aacaatatta tttaaccttt aattttgata atgaacctga aaatatttca 2940
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gtagataatc aaagattatt atctaca 3987

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&lt;210&gt; 130

&lt;211&gt; 3969

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3966)

&lt;223&gt; Sequence encoding BoNT/A-BD-PAR3-Xa

&lt;400&gt; 130

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## Li et al., Degradable Clostridial Toxins

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&lt;210&gt; 131

&lt;211&gt; 3987

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3984)

&lt;223&gt; Sequence encoding BoNT/A-BD-PAR4-Thrombin

&lt;400&gt; 131

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Li *et al.*, Degradable Clostridial Toxins

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## Li et al., Degradable Clostridial Toxins

```

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gtagataatc aaagattatt atctaca 3987

```

&lt;210&gt; 132

&lt;211&gt; 3969

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3966)

&lt;223&gt; Sequence encoding BoNT/A-BD-PAR4-Xa

&lt;400&gt; 132

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Li *et al.*, Degradable Clostridial Toxins

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&lt;210&gt; 133

&lt;211&gt; 6

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR1

&lt;400&gt; 133

Ser Phe Phe Leu Lys Asn

1

5

&lt;210&gt; 134

&lt;211&gt; 6

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(6)

&lt;223&gt; Hexapeptide comprising the tethered ligand of PAR3

&lt;400&gt; 134

Ser Phe Asn Gly Asn Glu

1

5

&lt;210&gt; 135

Li *et al.*, Degradable Clostridial Toxins

<211> 6  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> PEPTIDE  
 <222> (1)...(6)  
 <223> Hexapeptide comprising the tethered ligand of PAR4

<400> 135  
 Ser Phe Pro Gly Gln Pro  
 1 5

<210> 136  
 <211> 4053  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <221> mat\_peptide  
 <222> (1)...(4050)  
 <223> Expression optimized sequence encoding  
 BoNTA-ED-Par1thrombin

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 ctgctgacct ccacgttcg tggcatcccg ttctggggcg gtagcaccat cgacaccgaa 540  
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## Li et al., Degradable Clostridial Toxins

```

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&lt;210&gt; 137

&lt;211&gt; 4029

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4026)

<223> Expression optimized sequence encoding  
BoNTA-ED-PAR1FactorXa

&lt;400&gt; 137

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Li *et al.*, Degradable Clostridial Toxins

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ccgctgttaa						4029

Li *et al.*; Degradable Clostridial Toxins

&lt;211&gt; 4038

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4035)

&lt;223&gt; Expression optimized sequence encoding

BoNTA-ED-PAR2trypsin

&lt;400&gt; 138

```

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aacatcatca acacctccat cctgaacctg cgttacgaat ccaaccacct gatcgacctg 2820
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Li *et al.*, Degradable Clostridial Toxins

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tacttcaact ccatctccct gaacaacgaa tacaccatca ttaactgcat ggaaaaaac 3060
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ggcgaacgct cgctgtaa 4038

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&lt;210&gt; 139

&lt;211&gt; 4014

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4011)

&lt;223&gt; Expression optimized sequence encoding

BoNTA-ED-PAR2FactorXa

&lt;400&gt; 139

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aacaaaatct gggttatccc ggaacgtgac accttcacca acccggaaga aggcgacctg 300
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Li *et al.*, Degradable Clostridial Toxins

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&lt;210&gt; 140

&lt;211&gt; 4044

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4041)

<223> Expression optimized sequence encoding  
BoNTA-ED-PAR3thrombin

&lt;400&gt; 140

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Li *et al.*, Degradable Clostridial Toxins

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Li *et al.*, Degradable Clostridial Toxins

```

ggcatcacca acaagtgcaa aatgaacctg caggacaaca acggcaacga catcggcttc 3900
atcggcttcc accagttcaa caacatcgct aaactggttg cttccaactg gtacaaccgt 3960
cagatcgaac gttcctcccg taccctgggc tgctcctggg aattcatccc ggttgacgac 4020
ggctggggcg aacgtccgct gtaa                                     4044

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&lt;210&gt; 141

&lt;211&gt; 4020

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4017)

<223> Expression optimized sequence encoding  
BoNTA-ED-PAR3FactorXa

&lt;400&gt; 141

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## Li et al., Degradable Clostridial Toxins

```

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&lt;210&gt; 142

&lt;211&gt; 4071

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4068)

<223> Expression optimized sequence encoding  
BoNTA-ED-PAR4thrombin

&lt;400&gt; 142

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```



## - Li et al., Degradable Clostridial Toxins

```

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&lt;210&gt; 143

&lt;211&gt; 4047

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(4044)

<223> Expression optimized sequence encoding  
BoNTA-ED-PAR4FactorXa

Li *et al.*, Degradable Clostridial Toxins

&lt;400&gt; 143

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atcccggttc agctgtccaa atacgttgac aaccagcgct tgctgtccac cttcaccgaa 2760
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gcttccaaca acatcatggt caaactggac ggctgccgtg acaccaccg ttacatctgg 3360
atcaataact tcaacctggt cgacaaagaa ctgaacgaga aggaaatcaa agacctgtac 3420

```

## Li et al., Degradable Clostridial Toxins

```

gacaaccagt ccaactccgg catcctgaaa gacttctggg gcgactacct gcagtatgac 3480
aaaccgtact acatgctgaa cctgtacgac ccgaacaaat acgttgacgt taacaacgtt 3540
ggcatccgtg gctacatgta cctgaaaggg ccgctgggct ccgttatgac caccaacatc 3600
tacctgaact cctccctgta ccgtggcacc aaattcatca tcaagaagta cgcttccggc 3660
aacaagaca acatcgttcg taacaacgac cgtgtttaca tcaacgttgt agttaagaac 3720
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cagggcatca ccaacaagtg caaaatgaac ctgcaggaca acaacggcaa cgacatcggc 3900
ttcatcggct tccaccagtt caacaacatc gctaaactgg ttgcttccaa ctggtacaac 3960
cgtcagatcg aacgttcttc ccgtaccctg ggctgtcctt gggaattcat cccggttgac 4020
gacggctggg gcgaacgtcc gctgttaa

```

&lt;210&gt; 144

&lt;211&gt; 3921

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3918)

<223> Expression optimized sequence encoding  
BoNTA-TD-PAR1thrombin

&lt;400&gt; 144

```

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tacatcaaaa tcccgaacgc tggccagatg cagccgggta aagctttcaa aatccacaac 120
aaaatctggg taatcccggg acgtgacacc ttcaccaacc cggaagaagg cgacctgaac 180
ccgccgccgg aagctaaaca ggttccgggt tcctactacg actccaccta cctgtccacc 240
gacaacgaga aggacaacta cctgaaaggg gttaccaaac tgttcgaacg tatctactcc 300
accgacctgg gccgtatgct gctgacctcc atcgttctgt gcatcccggt ctggggcgggc 360
tccaccatcg acaccgaact gaaagttatc gacaccaact gcatcaacgt tatccagccg 420
gacggctcct accgttccga agaactgaac ctgggttatca tcggcccgct cgctgacatc 480
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ggctccaccc agtacatccg tttctccccg gacttcacct tcggcttcga agaatccctg 600
gaagttgaca ccaaccgctg gctggggcgt ggcaaattcg ctaccgaccc ggctgttacc 660
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cgtgttttca aagttaacac caacgcttac tacgaaatgt ccggcctgga agtttccttc 780
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aatccatcg ttggcaccac cgcttccctg cagtacatga agaacgtttt caaagagaag 960
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gtttacgact tcaccgacga aacctccgaa gtttccacca ccgacaaaat cgctgacatc 1920
accatcatta tcccgtaacat cggcccggtc ctgaacatcg gcaacatgct gtacaaagac 1980
gacttcgttg gcgctctgat cttctccggc gctgttatcc tgctggaatt catcccgga 2040
atcgctatcc cggttctggg caccttcgct ctgggttctt acatcgctaa caaagtctctg 2100

```

## Li et al., Degradable Clostridial Toxins

```

accgttcaga ccatcgacaa cgctctgtcc aaacgtaacg agaagtggga cgaagtttac 2160
aaatacatcg ttaccaactg gctggctaaa gttaacaccc agatcgacct gatccgtaag 2220
aagatgaaag aagctctgga gaaccaggct gaagctacca aagctatcat caactaccag 2280
tacaaccagt acaccgaaga ggaaaagaac aacatcaact tcaacatcga cgacctgtcc 2340
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tgctccggtt cctacctgat gaactccatg atcccgtacg gcgttaaacy tctggaagac 2460
ttcgacgctt ccctgaaaga cgctctgtct aaatacatct acgacaaccg tggcaccctg 2520
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ttccagctgt ccaaatacgt tgacaaccag cgtctgtctt ccaccttcac cgaatacatc 2640
aagaacatca tcaacacctc catcctgaac ctgctgtacg aatccaacca cctgatcgac 2700
ctgtcccgtt acgcttccaa gattaacatc ggctccaaag ttaacttcga ccgatcgac 2760
aagaaccaga tccagctgtt caacctggaa tcctccaaga ttgaagtatt cctgaagaac 2820
gctatcggtt acaactccat gtacgagaac ttctccacct ccttctggat ccgatcccg 2880
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aactccggct ggaaagtctt cctgaactac ggcgaaatca tctggaccct gcaggacacc 3000
caggaaatca aacagcgtgt tgtattttaa tactcccaga tgatcaacat ctccgactac 3060
atcaaccgtt ggatcttctg taccatcacc aacaaccgtc tgaacaactc caagatttac 3120
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taccgtctgg ctaccaacgc ttcccaggct ggcgttgaga agattctgtc cgctctggaa 3660
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atcaccacaa agtgcaagat gaacctgcag gacaacaacg gcaacgacat cggcttcatc 3780
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tggggcgaac gtccgctgta a 3921

```

&lt;210&gt; 145

&lt;211&gt; 3903

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3900)

&lt;223&gt; Expression optimized sequence encoding

BoNTA-TD-PAR1FactorXa

&lt;400&gt; 145

```

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gacaacgaga aggacaacta cctgaaaggc gttaccaaac tgttcgaacg tatctactcc 300
accgacctgg gccgtatgct gctgacctcc atcggttcgtg gcatcccggt ctggggcggc 360
tccaccatcg acaccgaact gaaagtatac gacaccaact gcatcaacgt tatccagccg 420
gacggctcct accgttccga agaactgaac ctgggttatca tcggcccgtc cgctgacatc 480
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ggctccaccc agtacatccg tttctccccg gacttcacct tcggcttcga agaatccctg 600
gaagttgaca ccaacccgct gctgggcgct ggcaaattcg ctaccgacct ggctgttacc 660
ctggctcagc aactgatcca cgtgggccac cgtctgtacg gcatcgctat caaccggaac 720
cgtgttttca aagttaacac caacgcttac tacgaaatgt ccggcctgga agtttccctt 780
gaagaactgc gtaccttcgg cggccacgac gctaaattca tcgactccct gcaggaaaac 840
gaattccgtc tgtactatta caacaaattc aaagacatcg cttccaccct gaacaaagct 900

```

Li *et al.*, Degradable Clostridial Toxins

```

aaatccatcg ttggcaccac cgcttcctcg cagtacatga agaacgtttt caaagagaag 960
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ctgtacaaaa tgctgaccga aatctacacc gaagacaact tcgttaaatt cttcaaagtt 1080
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taa 3903

```

&lt;210&gt; 146

&lt;211&gt; 3921

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

## Li et al., Degradable Clostridial Toxins

&lt;222&gt; (1)...(3918)

<223> Expression optimized sequence encoding  
BoNTA-TD-PAR2trypsin

&lt;400&gt; 146

```

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aaaatctggg taatcccggg acgtgacacc ttcaccaacc cggaagaagg cgacctgaac 180
ccgccgccgg aagctaaaca ggttccgggt tcctactacg actccaccta cctgtccacc 240
gacaacgaga aggacaacta cctgaaaggc gttaccaaac tggtcgaacg tatctactcc 300
accgacctgg gccgtatgct gctgacctcc atcgttcgtg gcatcccggt ctggggcggc 360
tccaccatcg acaccgaact gaaagtatat gacaccaact gcatcaacgt tatccagccg 420
gacggctcct accgttccga agaactgaac ctgggttatca tcggcccgct cgctgacatc 480
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ggctccaccc agtacatccg tttctctccg gacttcacct tcggcttcga agaattccctg 600
gaagttgaca ccaacccgct gctgggcgct ggcaaattcg ctaccgacct ggctgttacc 660
ctggctcacg aactgatcca cgtggccac cgtctgtacg gcatcgctat caaccggaac 720
cgtgtattta aagttaacac caacgcttac tacgaaatgt ccggcctgga agtttccttc 780
gaagaactgc gtaccttcgg cggccacgac gctaaattca tcgactccct gcaggaaaaac 840
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atcaaccggc gtctgatcga ccagaaaccg atctccaacc tgggcaacat ccacgttcc 3180
aacaacatca tgttcaaact ggacggctgc cgtgacacct accgttacat ctggatcaaa 3240

```

## Li et al., Degradable Clostridial Toxins

```

tacttcaacc tgttcgacaa agaactgaac gagaaggaaa tcaaagacct gtacgacaaac 3300
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tactacatgc tgaacctgta cgacccgaac aaatacgttg acgttaacaa cgttggcatc 3420
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atcgaacgtt cctcccgtag cctgggctgc tcctgggaat tcatcccggt tgacgacggc 3900
tggggcgaac gtccgctgta a 3921

```

&lt;210&gt; 147

&lt;211&gt; 3903

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3900)

&lt;223&gt; Expression optimized sequence encoding

BoNTA-TD-PAR2FactorXa

&lt;400&gt; 147

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aaaatctggg taatcccggg acgtgacacc ttcaccaacc cggaagaagg cgacctgaac 180
ccgcccggcg aagctaaaca gggtccgggt tcctactacg actccaccta cctgtccacc 240
gacaacgaga aggacaacta cctgaaaggc gttaccaaac tgttcgaacg tatctactcc 300
accgacctgg gccgtatgct gctgacctcc atcggttcgt gcatcccggt ctggggcggc 360
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```

Li *et al.*, Degradable Clostridial Toxins

```

atcttctccg gcgctgttat cctgctggaa ttcattcccg aaatcgctat cccggttctg 2040
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aacgctctgt ccaaacgtaa cgagaagtgg gacgaagttt acaaatacat cgttaccaac 2160
tggctggcta aagttaacac ccagatcgac ctgatccgta agaagatgaa agaagctctg 2220
gagaaccagg ctgaagctac caaagctatc atcaactacc agtacaacca gtacaccgaa 2280
gaggaaaaga acaacatcaa cttcaacatc gacgacctgt cctccaaact gaacgaatcc 2340
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aacatcgcta aactgggtgc ttccaactgg tacaaccgtc agatcgaacg ttctctccgt 3840
accctgggct gctcctggga attcatcccg gttgacgacg gctggggcga acgtccgctg 3900
taa 3903

```

&lt;210&gt; 148

&lt;211&gt; 3921

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3918)

<223> Expression optimized sequence encoding  
BoNTA-TD-PAR3thrombin

&lt;400&gt; 148

```

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tacatcaaaa tcccgaaacg tggccagatg cagccgggta aagctttcaa gattcacaac 120
aaaatctggg taatcccggg acgtgacacc ttaccaacc cggaagaagg cgacctgaac 180
ccgccgccgg aagctaaaca ggttccgggt tctactacg actccaccta cctgtccacc 240
gacaacgaga aggacaacta cctgaaaggc gttaccaaac tgttcgaacg tatctactcc 300
accgacctgg gccgtatgct gctgacctcc atcgttcgtg gcatcccgtt ctggggcggc 360
tccaccatcg acaccgaact gaaagttatc gacaccaact gcatcaacgt tatccagccg 420
gacggctcct accgttccga agaactgaac ctggttatca tcggcccgtc cgctgacatc 480
atccagttcg aatgcaaact cttcggccac gaagttctga acctgacctg taacggctac 540
ggctccaccc agtacatccg tttctctccg gacttcacct tcggcttcga agaatccctg 600
gaagttgaca ccaaccgctg gctgggcgct ggcaaattcg ctaccgacct ggctgttacc 660
ctggctcagc aactgatcca cgctggccac cgtctgtacg gcatcgctat caaccggaac 720
cgtgttttca aagttaacac caacgcttac tacgaaatgt ccggcctgga agtttccttc 780

```



## Li et al., Degradable Clostridial Toxins

```

gaagaactgc gtaccttcgg cggccacgac gctaaattca tcgactccct gcaggaaaac 840
gaattccgtc tgtactatta caacaaattc aaagacatcg cttccaccct gaacaaagct 900
aatccatcg ttggcaccac cgcttccctg cagtacatga agaacgtatt taaagagaag 960
tacctgctgt ccgaagacac ctccggcaaa ttctccgttg acaaaactgaa attcgacaaa 1020
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atcgaaacgtt cctcccgta cctgggctgc tctgggaaat tcatcccggg tgacgacggc 3900
tggggcgaac gtccgctgta a 3921

```

&lt;210&gt; 149

&lt;211&gt; 3903

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

Li *et al.*, Degradable Clostridial Toxins

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3900)

<223> Expression optimized sequence encoding  
BoNTA-TD-PAR3FactorXa

&lt;400&gt; 149

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```

Li *et al.*, Degradable Clostridial Toxins

```

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accctgggct gtcctggga attcatccc gttgacgacg gctggggcga acgtccgctg 3900
taa 3903

```

&lt;210&gt; 150

&lt;211&gt; 3921

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3918)

&lt;223&gt; Expression optimized sequence encoding

BoNTA-TD-PAR4thrombin

&lt;400&gt; 150

```

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aaaatctggg taatcccggga acgtgacacc ttcaccaacc cggagaagg cgacctgaac 180
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gtttacgact tcaccgacga aacctccgaa gtttccacca ccgacaaaat cgctgacatc 1920

```

Li *et al.*, Degradable Clostridial Toxins

```

accatcatta tcccgtacat cggcccggct ctgaacatcg gcaacatgct gtacaaagac 1980
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```

&lt;210&gt; 151

&lt;211&gt; 3903

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3900)

<223> Expression optimized sequence encoding  
BoNTA-TD-PAR4FactorXa

&lt;400&gt; 151

```

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tccaccatcg acaccgaact gaaagttatc gacaccaact gcatcaacgt tatccagccg 420
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## Li et al., Degradable Clostridial Toxins

```

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taa 3903

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<210> 152  
 <211> 3990  
 <212> DNA

## Li et al., Degradable Clostridial Toxins

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3987)

&lt;223&gt; Expression optimized sequence encoding

BoNTA-BD-PAR1Thrombin

&lt;400&gt; 152

```

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aaaatctggg taatcccggg acgtgacacc ttcaccaacc cggaagaagg cgacctgaac 180
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## Li et al., Degradable Clostridial Toxins

```

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```

&lt;210&gt; 153

&lt;211&gt; 3972

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3969)

<223> Expression optimized sequence encoding  
BoNTA-BD-PAR1FactorXa

&lt;400&gt; 153

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```

Li *et al.*, Degradable Clostridial Toxins

```

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ctgtccacct aa 3972

```

&lt;210&gt; 154

&lt;211&gt; 3990

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3987)

&lt;223&gt; Expression optimized sequence encoding

BoNTA-BD-PAR2trypsin

&lt;400&gt; 154

```

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## Li et al., Degradable Clostridial Toxins

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## Li et al., Degradable Clostridial Toxins

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3990

&lt;210&gt; 155

&lt;211&gt; 3972

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3969)

<223> Expression optimized sequence encoding  
BoNTA-BD-PAR2FactorXa

&lt;400&gt; 155

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Li *et al.*, Degradable Clostridial Toxins

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ctgtccacct aa 3972

```

&lt;210&gt; 156

&lt;211&gt; 3990

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3987)

<223> Expression optimized sequence encoding  
BoNTA-BD-PAR3thrombin

&lt;400&gt; 156

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LI *et al.*, Degradable Clostridial Toxins

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```

&lt;210&gt; 157

&lt;211&gt; 3972

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3969)

&lt;223&gt; Expression optimized sequence encoding

BoNTA-BD-PAR3FactorXa

&lt;400&gt; 157

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```

## Li et al., Degradable Clostridial Toxins

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## Li et al., Degradable Clostridial Toxins

```

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ctgtccacct aa 3972

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&lt;210&gt; 158

&lt;211&gt; 3990

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3987)

<223> Expression optimized sequence encoding  
BoNTA-BD-PAR4thrombin

&lt;400&gt; 158

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tccaccatcg acaccgaact gaaagtatat gacaccaact gcatcaacgt tatccagccg 420
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```

Li *et al.*, Degradable Clostridial Toxins

```

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gttgacaacc agcgtctgct gtccaccta 3990

```

&lt;210&gt; 159

&lt;211&gt; 3972

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (1)...(3969)

<223> Expression optimized sequence encoding  
BoNTA-BD-PAR4FactorXa

&lt;400&gt; 159

```

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Li *et al.*, Degradable Clostridial Toxins

```

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ctgtccacct aa 3972

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&lt;210&gt; 160

&lt;211&gt; 6

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)...(6)

<223> Variant of hexapeptide comprising the tethered  
ligand of PAR4



Li *et al.*, Degradable Clostridial Toxins

&lt;400&gt; 160

Ser Phe Pro Gly Gln Ala

1

5

A. CLASSIFICATION OF SUBJECT MATTER  
C07K14/33 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	US 2002/137886 A1 (LIN WEI-JEN ET AL) 26 September 2002 (2002-09-26) cited in the application page 2, column 1, paragraph 15 - paragraph 17	1-61
A	----- US 6 168 932 B1 (UCKUN FATIH M ET AL) 2 January 2001 (2001-01-02) column 1, line 23 - line 50	1-61
A	----- US 2003/124147 A1 (VALLERA DANIEL A ET AL) 3 July 2003 (2003-07-03) page 1, paragraph 5 - paragraph 6 ----- -/--	1-61

☒ Further documents are listed in the continuation of box C

☒ Patent family members are listed in annex.

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- \*X\* document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search

12 January 2006

Date of mailing of the international search report

24/01/2006

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Pérez-Mato, I

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	<p>PASTAN I ET AL: "PSEUDOMONAS EXOTOXIN: CHIMERIC TOXINS" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOCHEMICAL BIOLOGISTS, BIRMINGHAM,, US, vol. 264, no. 26, 15 September 1989 (1989-09-15), pages 15157-15160, XP000048580 ISSN: 0021-9258 page 15159, column 1, line 24 - page 15160, column 1, line 42</p>	1-61
A	<p>TSUI J K C: "BOTULINUM TOXIN AS A THERAPEUTIC AGENT" PHARMACOLOGY AND THERAPEUTICS, ELSEVIER, GB, vol. 72, no. 1, 1996, pages 13-24, XP001179766 ISSN: 0163-7258 page 20, column 2, line 4 - page 21, column 1, line 18</p>	1-61
A	<p>COUGHLIN S R: "Thrombin signalling and protease-activated receptors" NATURE, NATURE PUBLISHING GROUP, LONDON, GB, vol. 407, no. 6801, 14 September 2000 (2000-09-14), pages 258-264, XP002981969 ISSN: 0028-0836 the whole document</p>	1-61
A	<p>HOLLENBERG MORLEY D ET AL: "International Union of Pharmacology. XXVIII. Proteinase-activated receptors" PHARMACOLOGICAL REVIEWS, WILLIAMS AND WILKINS INC., BALTIMORE, MD,, US, vol. 54, no. 2, June 2002 (2002-06), pages 203-217, XP002234539 ISSN: 0031-6997 the whole document</p>	1-61

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